FUNCTIONAL MATERIALS AND NOVEL METHODS FOR THE FABRICATION OF MICROFLUIDIC DEVICES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is based on and claims priority to United States Provisional Patent Application Serial No. 60/544,905, filed February 13, 2004, which is incorporated herein by reference in its entirety.

GOVERNMENT INTEREST

This invention was made with U.S. Government support from Office of Naval Research No. N000140210185 and STC program of the National Science Foundation under Agreement No. CHE-9876674. The U.S. Government has certain rights in the invention.

TECHNICAL FIELD

The presently disclosed subject matter relates to functional materials and their use for fabricating and utilizing micro- and nano-scale devices.

ABBREVIATIONS

AC	=	alternating current
Ar	=	Argon
°C	=	degrees Celsius
cm	=	centimeter
8-CNVE	=	perfluoro(8-cyano-5-methyl-3,6-dioxa-1-octene)
CSM	=	cure site monomer
CTFE	=	chlorotrifluoroethylene
g	=	grams
h	=	hours
1-HPFP	=	1,2,3,3,3-pentafluoropropene
2-HPFP	=	1,1,3,3,3-pentafluoropropene
HFP	=	hexafluoropropylene
HMDS	=	hexamethyldisilazane

	IL	=	imprint lithography
	MCP	=	microcontact printing
	Me	=	methyl
	MEA	=	membrane electrode assembly
	MEMS	=	micro-electro-mechanical system
	MeOH	=	methanol
	MIMIC	=	micro-molding in capillaries
	mL	=	milliliters
	mm	=	millimeters
	mmol	=	millimoles
	M_n	=	number-average molar mass
	m.p.	=	melting point
	mW	=	milliwatts
	NCM	=	nano-contact molding
	NIL	=	nanoimprint lithography
	nm	=	nanometers
	Pd	=	palladium
	PAVE		perfluoro(alkyl vinyl) ether
	PDMS	=	poly(dimethylsiloxane)
	PEM	=	proton exchange membrane
	PFPE	=	perfluoropolyether
	PMVE		perfluoro(methyl vinyl) ether
	PPVE		perfluoro(propyl vinyl) ether
	PSEPVE	=	perfluoro-2-(2-fluorosulfonylethoxy)propyl vinyl ether
	PTFE	=	polytetrafluoroethylene
	SAMIM	=	solvent-assisted micro-molding
	SEM	=	scanning electron microscopy
	Si	=	silicon
	TFE	=	tetrafluoroethylene
	μm	=	micrometers
	UV	=	ultraviolet
	W	=	watts

ZDOL = poly(tetrafluoroethylene oxide-*co*-difluoromethylene oxide)α,ω diol

BACKGROUND

Microfluidic devices developed in the early 1990s were fabricated from hard materials, such as silicon and glass, using photolithography and etching techniques. See Ouellette, J., *The Industrial Physicist* **2003**, *August/September*, 14-17; Scherer, A., et al., *Science* **2000**, *290*, 1536-1539. Photolithography and etching techniques, however, are costly and labor intensive, require clean-room conditions, and pose several disadvantages from a materials standpoint. For these reasons, soft materials have emerged as alternative materials for microfluidic device fabrication. The use of soft materials has made possible the manufacture and actuation of devices containing valves, pumps, and mixers. See, e.g., Ouellette, J., *The Industrial Physicist* **2003**, *August/September*, 14-17; Scherer, A., et al., *Science* **2000**, *290*, 1536-1539; Unger, M. A., et al., *Science* **2000**, *288*, 113-116; McDonald, J. C., et al., *Acc. Chem. Res.* **2002**, *35*, 491-499; and Thorsen, T., et al., *Science* **2002**, *298*, 580-584. For example, one such microfluidic device allows for control over flow direction without the use of mechanical valves. See Zhao, B., et al., *Science* **2001**, *291*, 1023-1026.

The increasing complexity of microfluidic devices has created a demand to use such devices in a rapidly growing number of applications. To this end, the use of soft materials has allowed microfluidics to develop into a useful technology that has found application in genome mapping, rapid separations, sensors, nanoscale reactions, ink-jet printing, drug delivery, Lab-on-a-Chip, *in vitro* diagnostics, injection nozzles, biological studies, and drug screening. See, e.g., Ouellette, J., *The Industrial Physicist* **2003**, *August/September*, 14-17; Scherer, A., et al., *Science* **2000**, *290*, 1536-1539; Unger, M. A., et al., *Science* **2000**, *288*, 113-116; McDonald, J. C., et al., *Acc. Chem. Res.* **2002**, *35*, 491-499; Thorsen, T., et al., *Science* **2002**, *298*, 580-584; and Liu, J., et al., *Anal. Chem.* **2003**, *75*, 4718-4723.

Poly(dimethylsiloxane) (PDMS) is the soft material of choice for many microfluidic device applications. See Scherer, A., et al., *Science* **2000**, *290*,

1536-1539; Unger, M. A., et al., *Science* **2000**, *288*, 113-116; McDonald, J. C., et al., *Acc. Chem. Res.* **2002**, *35*, 491-499; Thorsen, T., et al., *Science* **2002**, *298*, 580-584; and Liu, J., et al., *Anal. Chem.* **2003**, *75*, 4718-4723. A PDMS material offers numerous attractive properties in microfluidic applications. Upon cross-linking, PDMS becomes an elastomeric material with a low Young's modulus, e.g., approximately 750 kPa. See Unger, M. A., et al., *Science* **2000**, *288*, 113-116. This property allows PDMS to conform to surfaces and to form reversible seals. Further, PDMS has a low surface energy, e.g., approximately 20 erg/cm^2, which can facilitate its release from molds after patterning. See Scherer, A., et al., *Science* **2000**, *290*, 1536-1539; McDonald, J. C., et al., *Acc. Chem. Res.* **2002**, *35*, 491-499.

Another important feature of PDMS is its outstanding gas permeability. This property allows gas bubbles within the channels of a microfluidic device to permeate out of the device. This property also is useful in sustaining cells and microorganisms inside the features of the microfluidic device. The nontoxic nature of silicones, such as PDMS, also is beneficial in this respect and allows for opportunities in the realm of medical implants. McDonald, J. C., et al., *Acc. Chem. Res.* **2002**, *35*, 491-499.

Many current PDMS microfluidic devices are based on SYLGARD® 184 (Dow Corning, Midland, Michigan, United States of America). SYLGARD® 184 is cured thermally through a platinum-catalyzed hydrosilation reaction. Complete curing of SYLGARD® 184 can take as long as five hours. The synthesis of a photocurable PDMS material, however, with mechanical properties similar to that of SYLGARD® 184 for use in soft lithography recently has been reported. See Choi, K. M., et al., *J. Am. Chem. Soc.* **2003**, *125*, 4060-4061.

Despite the aforementioned advantages, PDMS suffers from a drawback in microfluidic applications in that it swells in most organic solvents. Thus, PDMS-based microfluidic devices have a limited compatibility with various organic solvents. See Lee, J. N., et al., *Anal. Chem.* **2003**, *75*, 6544-6554. Among those organic solvents that swell PDMS are hexanes, ethyl ether, toluene, dichloromethane, acetone, and acetonitrile. See Lee, J. N., et al., *Anal. Chem.* **2003**, *75*, 6544-6554. The swelling of a PDMS microfluidic

device by organic solvents can disrupt its micron-scale features, e.g., a channel or plurality of channels, and can restrict or completely shut off the flow of organic solvents through the channels. Thus, microfluidic applications with a PDMS-based device are limited to the use of fluids, such as water, that do not swell PDMS. As a result, those applications that require the use of organic solvents likely will need to use microfluidic systems fabricated from hard materials, such as glass and silicon. See Lee, J. N., et al., *Anal. Chem.* **2003**, *75*, 6544-6554. This approach, however, is limited by the disadvantages of fabricating microfluidic devices from hard materials.

Moreover, PDMS-based devices and materials are notorious for not being adequately inert enough to allow them to be used even in aqueous-based chemistries. For example, PDMS is susceptible to reaction with weak and strong acids and bases. PDMS-based devices also are notorious for containing extractables, in particular extractable oligomers and cyclic siloxanes, especially after exposure to acids and bases. Because PDMS is easily swollen by organics, hydrophobic materials, even those hydrophobic materials that are slightly soluble in water, can partition into PDMS-based materials used to construct PDMS-based microfluidic devices.

Thus, an elastomeric material that exhibits the attractive mechanical properties of PDMS combined with a resistance to swelling in common organic solvents would extend the use of microfluidic devices to a variety of new chemical applications that are inaccessible by current PDMS-based devices. Accordingly, the approach demonstrated by the presently disclosed subject matter uses an elastomeric material, more particularly a functional perfluoropolyether (PFPE) material, which is resistant to swelling in common organic solvents to fabricate a microfluidic device.

Functional PFPE materials are liquids at room temperature, exhibit low surface energy, low modulus, high gas permeability, and low toxicity with the added feature of being extremely chemically resistant. See Scheirs, J., *Modern Fluoropolymers*; John Wiley & Sons, Ltd.: New York, 1997; pp 435-485. Further, PFPE materials exhibit hydrophobic and lyophobic properties. For this reason, PFPE materials are often used as lubricants on high-performance machinery operating in harsh conditions. The synthesis and

solubility of PFPE materials in supercritical carbon dioxide has been reported. See Bunyard, W., et al., *Macromolecules* **1999**, *32*, 8224-8226. Beyond PFPEs, fluoroelastomers also can comprise fluoroolefin-based materials, including, but not limited to, copolymers of tetrafluoroethylene, hexafluoropropylene, vinylidene fluoride and alkyl vinyl ethers, often with additional cure site monomers added for crosslinking.

A PFPE microfluidic device has been previously reported by Rolland, J. et al. *JACS* **2004**, *126*, 2322-2323. The device was fabricated from a functionalized PFPE material (e.g., a PFPE dimethacrylate (MW = 4,000 g/mol)) having a viscosity of the functionalized material of approximately 800 cSt. This material was end-functionalized with a free radically polymerizable methacrylate group and UV photocured free radically with a photoinitiator. In Rolland, J. et al., *supra*, multilayer PFPE devices were generated using a specific partial UV curing technique and the adhesion was weak and generally not strong enough for a wide range of applications. Further, the adhesion technique described by Rolland, J. et al. did not provide for adhesion to other substrates such as glass.

The presently disclosed subject matter describes the use of fluoroelastomers, especially a functional perfluoropolyether as a material for fabricating a solvent-resistant micro-and nano-scale structures, such as a microfluidic device. The use of fluoroelastomers and functional perfluoropolyethers in particular as materials for fabricating a microfluidic device addresses the problems associated with swelling in organic solvents exhibited by microfluidic devices made from other polymeric materials, such as PDMS. Accordingly, PFPE-based microfluidic devices can be used to control the flow of a small volume of a fluid, such as an organic solvent, and to perform micro- and nano-scale chemical reactions that are not amenable to other polymeric microfluidic devices.

SUMMARY

The presently disclosed subject matter provides functional perfluoropolyether (PFPE) materials for use in fabricating microfluidic devices. In some embodiments, the presently disclosed subject matter provides a

method for adhering two-dimensional and three-dimensional micro- and/or nano-scale structures, e.g., a microfluidic network, to a substrate. Further, in some embodiments, the presently disclosed subject matter provides a method for forming a hybrid microfluidic device, for example, a microfluidic device comprising a perfluoropolyether layer adhered to a second polymeric layer, wherein the second polymeric layer comprises, for example, a poly(dimethylsiloxane) layer.

The presently disclosed subject matter also provides methods for fabricating a micro- and/or nano-scale structure, e.g., a microfluidic device, by using sacrificial layers of a degradable material. More particularly, the presently disclosed subject matter provides a method for fabricating micro- and/or nano-scale structures using degradable or selectively soluble polymers as scaffolds for producing complex, two-dimensional (2-D) and three-dimensional (3-D) microfluidic networks.

Further, the presently disclosed subject matter provides functional materials for use in attaching biological and other "switchable" molecules to the interior surface of a microfluidic channel. For example, attaching a biomolecule, such as a biopolymer, to the interior surface of a microfluidic channel, provides for combinatorial peptide synthesis and/or rapid screening of enzyme-protein interactions. Further, lining a microfluidic channel with a catalyst, allows for rapid catalyst screening. Also, introduction of a switchable organic molecule into a microfluidic channel allows for the fabrication of microfluidic devices comprising hydrophilic channels and hydrophobic channels.

In some embodiments, the presently disclosed subject matter provides a method for using a functionalized perfluoropolyether network as a gas separation membrane.

Accordingly, it is an object of the presently disclosed subject matter to provide functional perfluoropolyether materials for use in fabricating and utilizing micro- and nano-scale devices, including microfluidic devices. This and other objects are achieved in whole or in part by the presently disclosed subject matter.

An object of the presently disclosed subject matter having been stated

hereinabove, other aspects and objects will become evident as the description proceeds when taken in connection with the accompanying Drawings and Examples as best described herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C are a series of schematic end views depicting the formation of a patterned layer of polymeric material in accordance with the presently disclosed subject matter.

Figures 2A-2D are a series of schematic end views depicting the formation of a microfluidic device comprising two patterned layers of a polymeric material in accordance with the presently disclosed subject matter.

Figures 3A-3C are schematic representations of an embodiment of the presently disclosed method for adhering a functional microfluidic device to a treated substrate.

Figures 4A-4C are schematic representations of an embodiment of the presently disclosed method for fabricating a multilayer microfluidic device.

Figures 5A and 5B are schematic representations of an embodiment of the presently disclosed method for functionalizing the interior surface of a microfluidic channel and the surface of a microtiter well.

Figure 5A is a schematic representation of an embodiment of the presently disclosed method for functionalizing the interior surface of a microfluidic channel.

Figure 5B is a schematic representation of an embodiment of the presently disclosed method for functionalizing the surface of a microtiter well.

Figures 6A-6D are schematic representations of an embodiment of the presently disclosed method for fabricating a microstructure using a degradable and/or selectively soluble material.

Figures 7A-7C are schematic representations of an embodiment of the presently disclosed method for fabricating complex structures in a micro- and/or nano-scale device using degradable and/or selectively soluble materials.

Figure 8 is a schematic plan view of a microfluidic device in accordance with the presently disclosed subject matter.

Figure 9 is a schematic of an integrated microfluidic system for biopolymer synthesis.

Figure 10 is schematic view of a system for flowing a solution or conducting a chemical reaction in a microfluidic device in accordance with the presently disclosed subject matter. The microfluidic device **800** is depicted as a schematic plan view as shown in Figure 8.

DETAILED DESCRIPTION

The presently disclosed subject matter provides materials and methods for use in forming a microfluidic device and for imparting chemical functionality to a microfluidic device. In some embodiments, the presently disclosed methods comprise introducing chemical functionalities that promote and/or increase the adhesion between the layers of the microfluidic device to one another. In some embodiments, the chemical functionalities promote and/or increase the adhesion between a layer of the microfluidic device and another surface. Accordingly, in some embodiments, the presently disclosed subject matter provides a method for adhering two-dimensional and three-dimensional microfluidic networks to a substrate. In some embodiments, the presently disclosed method allows for bonding a perfluoropolyether (PFPE) material to other materials, such as a poly(dimethyl siloxane) (PDMS) material, a polyurethane material, a silicone-containing polyurethane material, and a PFPE-PDMS block copolymer material. Thus, in some embodiments, the presently disclosed subject matter provides a method for forming a hybrid microfluidic device, for example, a microfluidic device comprising a perfluoropolyether layer adhered to a polydimethylsiloxane layer, a polyurethane layer, a silicone-containing polyurethane layer, and a PFPE-PDMS block copolymer layer.

In some embodiments, the method comprises introducing a chemical functionality to the interior surface of a microfluidic channel and/or a microtiter well. In some embodiments, the introduction of a chemical functionality to the interior surface of the microfluidic channel and/or microtiter well provides for the attachment of a biopolymer and other small organic "switchable" molecules that can affect the hydrophobicity or the reactivity of the microfluidic

channel and/or microtiter well.

In some embodiments, the presently disclosed subject matter provides a method for forming a micro- and/or nano-scale structure in which scaffolds of degradable or selectively soluble polymers are used to form channels, for example, inside a microfluidic device. Accordingly, the molding method disclosed herein allows for complex three-dimensional networks of microfluidic channels to be formed in a one step process.

In some embodiments, the presently disclosed subject matter provides a method for using a functionalized perfluoropolyether network as a gas separation membrane.

The presently disclosed subject matter will now be described more fully hereinafter with reference to the accompanying Drawings and Examples, in which representative embodiments are shown. The presently disclosed subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the embodiments to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Throughout the specification and claims, a given chemical formula or name shall encompass all optical and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

I. Definitions

As used herein, the term "microfluidic device" generally refers to a device through which materials, particularly fluid borne materials, such as liquids, can be transported, in some embodiments on a micro-scale, and in some embodiments on a nano-scale. Thus, the microfluidic devices described by the presently disclosed subject matter can comprise microscale

features, nanoscale features, and combinations thereof.

Accordingly, a microfluidic device typically comprises structural or functional features dimensioned on the order of a millimeter-scale or less, which are capable of manipulating a fluid at a flow rate on the order of a microliter/min or less. Typically, such features include, but are not limited to channels, fluid reservoirs, reaction chambers, mixing chambers, and separation regions. In some examples, the channels include at least one cross-sectional dimension that is in a range of from about 0.1 μm to about 500 μm. The use of dimensions on this order allows the incorporation of a greater number of channels in a smaller area, and utilizes smaller volumes of fluids.

A microfluidic device can exist alone or can be a part of a microfluidic system which, for example and without limitation, can include: pumps for introducing fluids, e.g., samples, reagents, buffers and the like, into the system and/or through the system; detection equipment or systems; reagent, product or data storage systems; and control systems for controlling fluid transport and/or direction within the device, monitoring and controlling environmental conditions to which fluids in the device are subjected, e.g., temperature, current, and the like.

As used herein, the term "device" includes, but is not limited to, a microfluidic device, a microtiter plate, tubing, a hose, and the like.

As used herein, the terms "channel," "microscale channel," and "microfluidic channel" are used interchangeably and can mean a recess or cavity formed in a material by imparting a pattern from a patterned substrate into a material or by any suitable material removing technique, or can mean a recess or cavity in combination with any suitable fluid-conducting structure mounted in the recess or cavity, such as a tube, capillary, or the like.

As used herein, the terms "flow channel" and "control channel" are used interchangeably and can mean a channel in a microfluidic device in which a material, such as a fluid, e.g., a gas or a liquid, can flow through. More particularly, the term "flow channel" refers to a channel in which a material of interest, e.g., a solvent or a chemical reagent, can flow through. Further, the term "control channel" refers to a flow channel in which a material, such as a fluid, e.g., a gas or a liquid, can flow through in such a way

to actuate a valve or pump.

As used herein, the term "valve" unless otherwise indicated refers to a configuration in which two channels are separated by an elastomeric segment, e.g., a PFPE segment that can be deflected into or retracted from one of the channels, e.g., a flow channel, in response to an actuation force applied to the other channel, e.g., a control channel. The term "valve" also includes one-way valves, which comprise channels separated by a bead.

As used herein, the term "pattern" can mean a channel or a microfluidic channel or an integrated network of microfluidic channels, which, in some embodiments, can intersect at predetermined points. A pattern also can comprise one or more of a micro- or nano-scale fluid reservoir, a micro- or nano-scale reaction chamber, a micro- or nano-scale mixing chamber, and a micro- or nano-scale separation region.

As used herein, the term "intersect" can mean to meet at a point, to meet at a point and cut through or across, or to meet at a point and overlap. More particularly, as used herein, the term "intersect" describes an embodiment wherein two channels meet at a point, meet at a point and cut through or across one another, or meet at a point and overlap one another. Accordingly, in some embodiments, two channels can intersect, i.e., meet at a point or meet at a point and cut through one another, and be in fluid communication with one another. In some embodiments, two channels can intersect, i.e., meet at a point and overlap one another, and not be in fluid communication with one another, as is the case when a flow channel and a control channel intersect.

As used herein, the term "communicate" (e.g., a first component "communicates with" or "is in communication with" a second component) and grammatical variations thereof are used to indicate a structural, functional, mechanical, electrical, optical, or fluidic relationship, or any combination thereof, between two or more components or elements. As such, the fact that one component is said to communicate with a second component is not intended to exclude the possibility that additional components can be present between, and/or operatively associated or engaged with, the first and second components.

In referring to the use of a microfluidic device for handling the containment or movement of fluid, the terms "in", "on", "into", "onto", "through", and "across" the device generally have equivalent meanings.

As used herein, the term "monolithic" refers to a structure comprising or acting as a single, uniform structure.

As used herein, the term "non-biological organic materials" refers to organic materials, i.e., those compounds having covalent carbon-carbon bonds, other than biological materials. As used herein, the term "biological materials" includes nucleic acid polymers (e.g., DNA, RNA) amino acid polymers (e.g., enzymes, proteins, and the like) and small organic compounds (e.g., steroids, hormones) wherein the small organic compounds have biological activity, especially biological activity for humans or commercially significant animals, such as pets and livestock, and where the small organic compounds are used primarily for therapeutic or diagnostic purposes. While biological materials are of interest with respect to pharmaceutical and biotechnological applications, a large number of applications involve chemical processes that are enhanced by other than biological materials, i.e., non-biological organic materials.

As used herein, the term "partial cure" refers to a process wherein less than about %100 of the polymerizable groups are reacted. Thus, the term "partially-cured material" refers to a material which has undergone a partial cure process.

As used herein, the term "full cure" refers to a process wherein about 100% of the polymerizable groups are reacted. Thus, the term "fully-cured material" refers to a material which has undergone a full cure process.

Following long-standing patent law convention, the terms "a", "an", and "the" refer to "one or more" when used in this application, including the claims. Thus, for example, reference to "a microfluidic channel" includes a plurality of such microfluidic channels, and so forth.

II. Materials

The presently disclosed subject matter broadly describes and employs solvent resistant, low surface energy polymeric materials, especially derived from casting liquid PFPE precursor materials onto a patterned substrate and then curing the liquid PFPE precursor materials to generate a patterned layer of functional PFPE material, which can be used to form a microfluidic device.

Representative solvent resistant elastomer-based materials include but are not limited to fluorinated elastomer-based materials. As used herein, the term "solvent resistant" refers to a material, such as an elastomeric material that neither swells nor dissolves in common hydrocarbon-based organic solvents or acidic or basic aqueous solutions. Representative fluorinated elastomer-based materials include but are not limited to perfluoropolyether (PFPE)-based materials.

Functional liquid PFPE materials exhibit desirable properties for use in a microfluidic device. For example, functional PFPE materials typically have a low surface energy (for example, about 12 mN/m); are non-toxic, UV and visible light transparent, and highly gas permeable; and cure into a tough, durable, highly fluorinated elastomeric or glassy materials with excellent release properties and resistance to swelling. The properties of these materials can be tuned over a wide range through the judicious choice of additives, fillers, reactive co-monomers, and functionalization agents. Such properties that are desirable to modify, include, but are not limited to, modulus, tear strength, surface energy, permeability, functionality, mode of cure, solubility and swelling characteristics, and the like. The non-swelling nature and easy release properties of the presently disclosed PFPE materials allow for the fabrication of microfluidic devices.

II.A. Perfluoropolyether Materials Prepared from a Liquid PFPE Precursor Material Having a Viscosity Less Than About 100 Centistokes.

As would be recognized by one of ordinary skill in the art, perfluoropolyethers (PFPEs) have been in use for over 25 years for many applications. Commercial PFPE materials are made by polymerization of

perfluorinated monomers. The first member of this class was made by the cesium fluoride catalyzed polymerization of hexafluoropropene oxide (HFPO) yielding a series of branched polymers designated as KRYTOX® (DuPont, Wilmington, Delaware, United States of America). A similar polymer is produced by the UV catalyzed photo-oxidation of hexafluoropropene (FOMBLIN® Y) (Solvay Solexis, Brussels, Belgium). Further, a linear polymer (FOMBLIN® Z) (Solvay) is prepared by a similar process, but utilizing tetrafluoroethylene. Finally, a fourth polymer (DEMNUM®) (Daikin Industries, Ltd., Osaka, Japan) is produced by polymerization of tetrafluorooxetane followed by direct fluorination. Structures for these fluids are presented in Table I. Table II contains property data for some members of the PFPE class of lubricants. Likewise, the physical properties of functional PFPEs are provided in Table III. In addition to these commercially available PFPE fluids, a new series of structures are being prepared by direct fluorination technology. Representative structures of these new PFPE materials appear in Table IV. Of the abovementioned PFPE fluids, only KRYTOX® and FOMBLIN® Z have been extensively used in applications. See Jones, W. R., Jr., The Properties of Perfluoropolyethers Used for Space Applications, NASA Technical Memorandum 106275 (July 1993), which is incorporated herein by reference in its entirety. Accordingly, the use of such PFPE materials is provided in the presently disclosed subject matter.

Table I. Names and Chemical Structures of Commercial PFPE Fluids

Name	Structure
DEMNUM®	$C_3F_7O(CF_2CF_2CF_2O)_xC_2F_5$
KRYTOX®	$C_3F_7O[CF(CF_3)CF_2O]_xC_2F_5$
FOMBLIN® Y	$C_3F_7O[CF(CF_3)CF_2O]_x(CF_2O)_yC_2F_5$
FOMBLIN® Z	$CF_3O(CF_2CF_2O)_x(CF_2O)_yCF_3$

Table II. PFPE Physical Properties

Lubricant	Average Molecular Weight	Viscosity at 20 °C, (cSt)	Viscosity Index	Pour Point, °C	Vapor Pressure, Torr	
					20 °C	100 °C
FOMBLIN® Z-25	9500	255	355	-66	$2.9x10^{-12}$	$1x10^{-8}$
KRYTOX® 143AB	3700	230	113	-40	$1.5x10^{-6}$	$3x10^{-4}$
KRYTOX® 143AC	6250	800	134	-35	$2x10^{-8}$	$8x10^{-6}$
DEMNUM® S-200	8400	500	210	-53	$1x10^{-10}$	$1x10^{-7}$

Table III. PFPE Physical Properties of Functional PFPEs

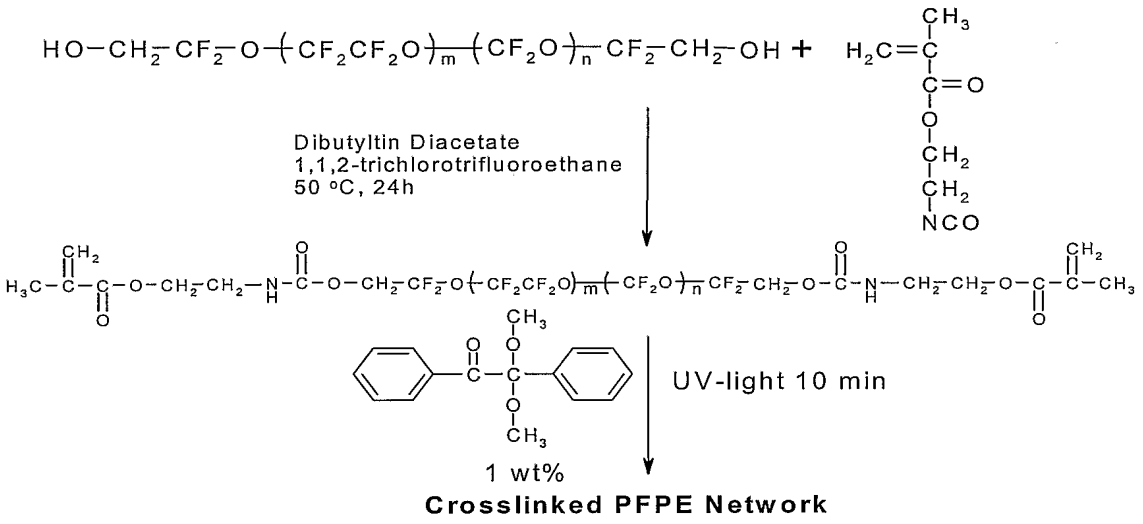
Lubricant	Average Molecular Weight	Viscosity at 20 °C, (cSt)	Vapor Pressure, Torr	
			20 °C	100 °C
FOMBLIN® Z-DOL 2000	2000	85	$2.0x10^{-5}$	$2.0x10^{-5}$
FOMBLIN® Z-DOL 2500	2500	76	$1.0x10^{-7}$	$1.0x10^{-4}$
FOMBLIN® Z-DOL 4000	4000	100	$1.0x10^{-8}$	$1.0x10^{-4}$
FOMBLIN® Z-TETROL	500	2000	$5.0x10^{-7}$	$2.0x10^{-4}$

Table IV. Names and Chemical Structures of Representative PFPE Fluids

Name	Structure[a]
Perfluoropoly(methylene oxide) (PMO)	$CF_3O(CF_2O)_xCF_3$
Perfluoropoly(ethylene oxide) (PEO)	$CF_3O(CF_2CF_2O)_xCF_3$
Perfluoropoly(dioxolane) (DIOX)	$CF_3O(CF_2CF_2OCF_2O)_xCF_3$
Perfluoropoly(trioxocane) (TRIOX)	$CF_3O[(CF_2CF_2O)_2CF_2O]_xCF_3$

[a] wherein x is any integer.

In some embodiments, the perfluoropolyether precursor comprises poly(tetrafluoroethylene oxide-co-difluoromethylene oxide)α,ω diol, which in some embodiments can be photocured to form one of a perfluoropolyether dimethacrylate and a perfluoropolyether distyrenic compound. A representative scheme for the synthesis and photocuring of a functionalized perfluoropolyether is provided in Scheme 1.



Scheme 1. Synthesis and Photocuring of Functionalized Perfluoropolyethers.

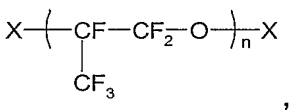
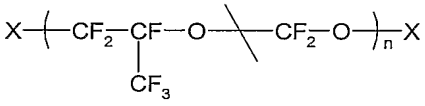
II.B. Perfluoropolyether Materials Prepared from a Liquid PFPE Precursor Material Having a Viscosity Greater Than About 100 Centistokes.

The methods provided herein below for promoting and/or increasing adhesion between a layer of a PFPE material and another material and/or a substrate and for adding a chemical functionality to a surface comprise a PFPE material having a characteristic selected from the group consisting of a viscosity greater than about 100 centistokes (cSt) and a viscosity less than

about 100 cSt, provided that the liquid PFPE precursor material having a viscosity less than 100 cSt is not a free-radically photocurable PFPE material. As provided herein, the viscosity of a liquid PFPE precursor material refers to the viscosity of that material prior to functionalization, e.g., functionalization with a methacrylate or a styrenic group.

Thus, in some embodiments, PFPE material is prepared from a liquid PFPE precursor material having a viscosity greater than about 100 centistokes (cSt). In some embodiments, the liquid PFPE precursor is end-capped with a polymerizable group. In some embodiments, the polymerizable group is selected from the group consisting of an acrylate, a methacrylate, an epoxy, an amino, a carboxylic, an anhydride, a maleimide, an isocyanato, an olefinic, and a styrenic group.

In some embodiments, the perfluoropolyether material comprises a backbone structure selected from the group consisting of:


,

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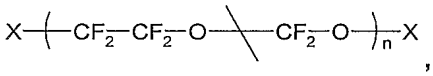
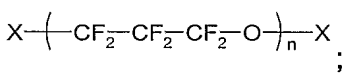
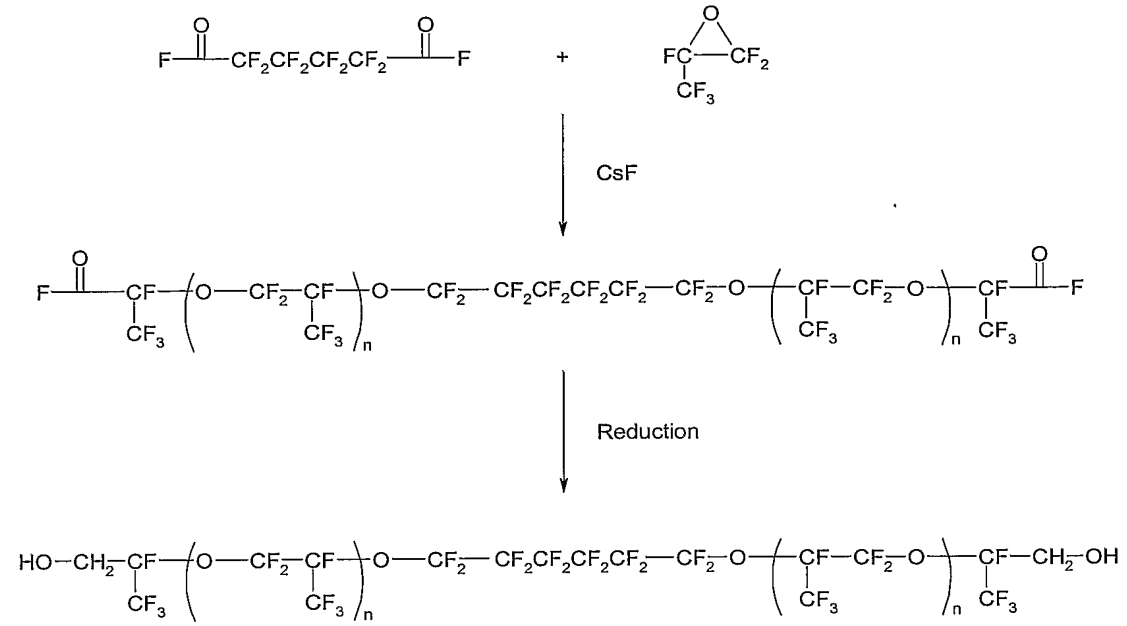

, and

;

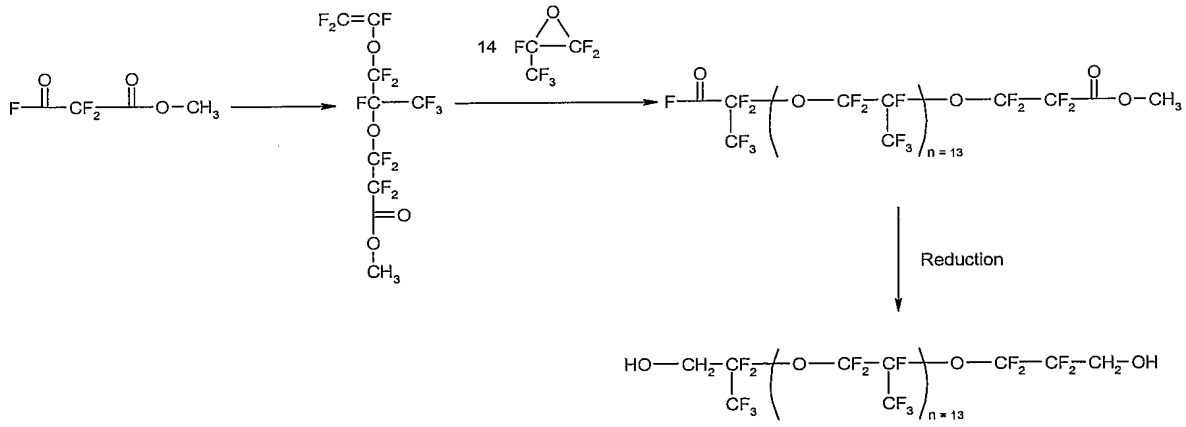
wherein X is present or absent, and when present comprises an endcapping group, and n is an integer from 1 to 100.

In some embodiments, the PFPE liquid precursor is synthesized from hexafluoropropylene oxide as shown in Scheme 2.



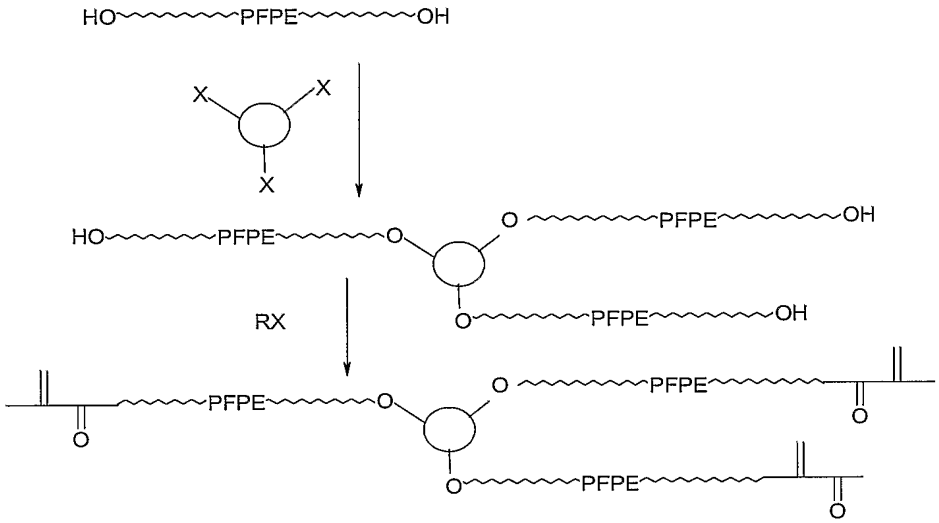
Scheme 2. Synthesis of a liquid PFPE precursor material from hexafluoropropylene oxide.

Is some embodiments, the liquid PFPE precursor is synthesized from hexafluoropropylene oxide as shown in Scheme 3.



Scheme 3. Synthesis of a liquid PFPE precursor material from hexafluoropropylene oxide.

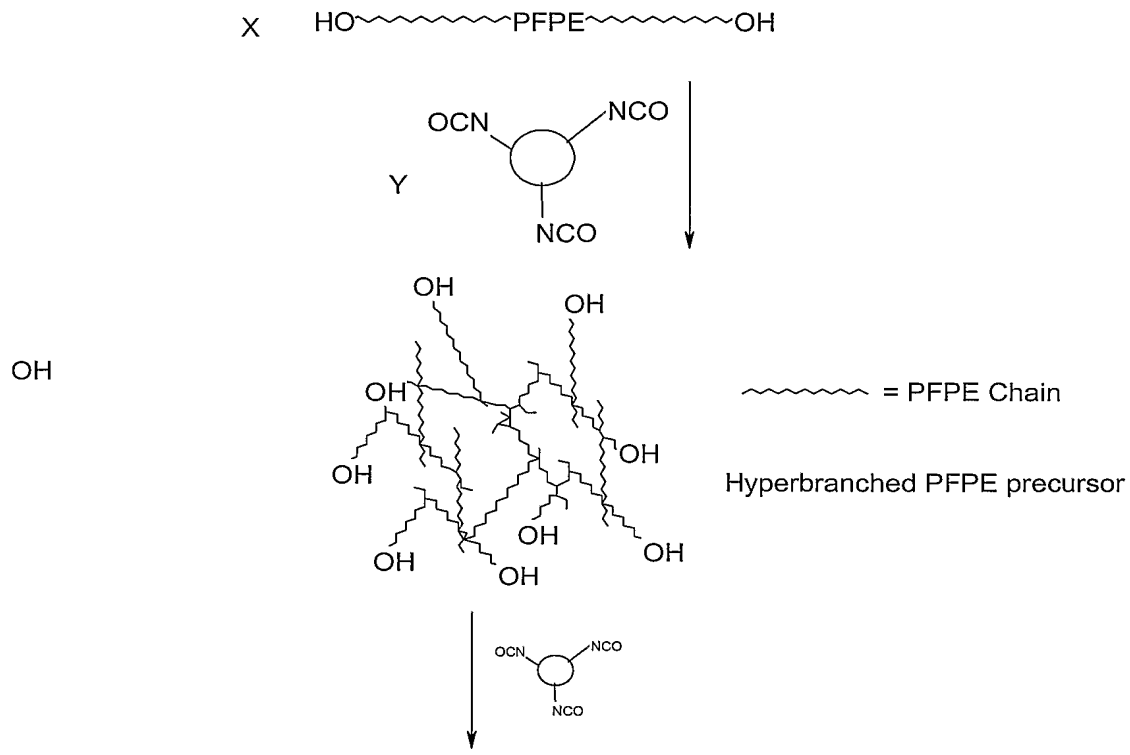
In some embodiments the liquid PFPE precursor comprises a chain extended material such that two or more chains are linked together before adding polymerizablable groups. Accordingly, in some embodiments, a "linker group" joins two chains to one molecule. In some embodiments, as shown in Scheme 4, the linker group joins three or more chains.



Scheme 4. Linker group joining three PFPE chains.

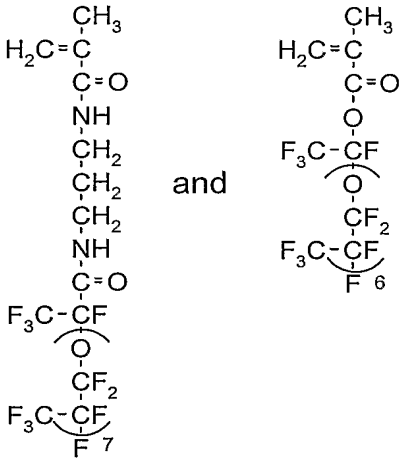
In some embodiments, X is selected from the group consisting of an isocyanate, an acid chloride, an epoxy, and a halogen. In some embodiments, R is selected from the group consisting of an acrylate, a methacrylate, a styrene, an epoxy, a carboxylic, an anhydride, a maleimide, an isocyanate, an olefinic, and an amine. In some embodiments, the circle represents any multifunctional molecule. In some embodiments, the multifunctional molecule comprises a cyclic molecule. PFPE refers to any PFPE material provided hereinabove.

In some embodiments, the liquid PFPE precursor comprises a hyperbranched polymer as provided in Scheme 5, wherein PFPE refers to any PFPE material provided hereinabove.



Scheme 5. Hyperbranched PFPE liquid precursor material.

In some embodiments, the liquid PFPE material comprises an end-functionalized material selected from the group consisting of:

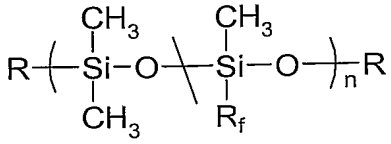


In some embodiments the PFPE liquid precursor is encapped with an epoxy moiety that can be photocured using a photoacid generator. Photoacid generators suitable for use in the presently disclosed subject matter include,

but are not limited to: bis(4-*tert*-butylphenyl)iodonium *p*-toluenesulfonate, bis(4-*tert*-butylphenyl)iodonium triflate, (4-bromophenyl)diphenylsulfonium triflate, (*tert*-butoxycarbonylmethoxynaphthyl)-diphenylsulfonium triflate, (*tert*-butoxycarbonylmethoxyphenyl)diphenylsulfonium triflate, (4-*tert*-butylphenyl)diphenylsulfonium triflate, (4-chlorophenyl)diphenylsulfonium triflate, diphenyliodonium-9,10-dimethoxyanthracene-2-sulfonate, diphenyliodonium hexafluorophosphate, diphenyliodonium nitrate, diphenyliodonium perfluoro-1-butanesulfonate, diphenyliodonium *p*-toluenesulfonate, diphenyliodonium triflate, (4-fluorophenyl)diphenylsulfonium triflate, *N*-hydroxynaphthalimide triflate, *N*-hydroxy-5-norbornene-2,3-dicarboximide perfluoro-1-butanesulfonate, *N*-hydroxyphthalimide triflate, [4-[(2-hydroxytetradecyl)oxy]phenyl]phenyliodonium hexafluoroantimonate, (4-iodophenyl)diphenylsulfonium triflate, (4-methoxyphenyl)diphenylsulfonium triflate, 2-(4-methoxystyryl)-4,6-bis(trichloromethyl)-1,3,5-triazine, (4-methylphenyl)diphenylsulfonium triflate, (4-methylthiophenyl)methyl phenyl sulfonium triflate, 2-naphthyl diphenylsulfonium triflate, (4-phenoxyphenyl)diphenylsulfonium triflate, (4-phenylthiophenyl)diphenylsulfonium triflate, thiobis(triphenyl sulfonium hexafluorophosphate), triarylsulfonium hexafluoroantimonate salts, triarylsulfonium hexafluorophosphate salts, triphenylsulfonium perfluoro-1-butanesufonate, triphenylsulfonium triflate, tris(4-*tert*-butylphenyl)sulfonium perfluoro-1-butanesulfonate, and tris(4-*tert*-butylphenyl)sulfonium triflate.

In some embodiments the liquid PFPE precursor cures into a highly UV and/or highly visible light transparent elastomer. In some embodiments the liquid PFPE precursor cures into an elastomer that is highly permeable to oxygen, carbon dioxide, and nitrogen, a property that can facilitate maintaining the viability of biological fluids/cells disposed therein. In some embodiments, additives are added or layers are created to enhance the barrier properties of the device to molecules, such as oxygen, carbon dioxide, nitrogen, dyes, reagents, and the like.

In some embodiments, the material suitable for use with the presently disclosed subject matter comprises a silicone material comprising a fluoroalkyl functionalized polydimethylsiloxane (PDMS) having the following structure:



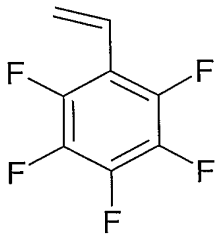
wherein:

R is selected from the group consisting of an acrylate, a methacrylate, and a vinyl group;

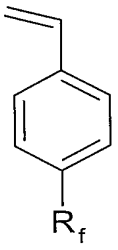
R_f comprises a fluoroalkyl chain; and

n is an integer from 1 to 100,000.

In some embodiments, the material suitable for use with the presently disclosed subject matter comprises a styrenic material comprising a fluorinated styrene monomer selected from the group consisting of:



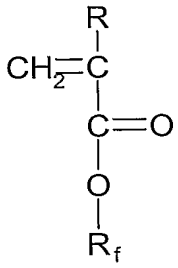
and



,

wherein R_f comprises a fluoroalkyl chain.

In some embodiments, the material suitable for use with the presently disclosed subject matter comprises an acrylate material comprising a fluorinated acrylate or a fluorinated methacrylate having the following structure:



wherein:

R is selected from the group consisting of H, alkyl, substituted alkyl, aryl, and substituted aryl; and

R_f comprises a fluoroalkyl chain with a $-CH_2-$ or a $-CH_2-CH_2-$ spacer between a perfluoroalkyl chain and the ester linkage. In some embodiments, the perfluoroalkyl group has hydrogen substituents.

In some embodiments, the material suitable for use with the presently disclosed subject matter comprises a triazine fluoropolymer comprising a fluorinated monomer.

In some embodiments, the fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction comprises a functionalized olefin. In some embodiments, the functionalized olefin comprises a functionalized cyclic olefin.

II.C. Fluoroolefin-based Materials

Further, in some embodiments, the materials used herein are selected from highly fluorinated fluoroelastomers, e.g., fluoroelastomers comprising at least fifty-eight weight percent fluorine, as described in U.S. Patent No. 6,512,063 to Tang, which is incorporated herein by reference in its entirety. Such fluoroelastomers can be partially fluorinated or perfluorinated and can contain between 25 to 70 weight percent, based on the weight of the fluoroelastomer, of copolymerized units of a first monomer, e.g., vinylidene fluoride (VF_2) or tetrafluoroethylene (TFE). The remaining units of the fluoroelastomers comprise one or more additional copolymerized monomers, which are different from the first monomer, and are selected from the group consisting of fluorine-containing olefins, fluorine containing vinyl ethers, hydrocarbon olefins, and combinations thereof.

These fluoroelastomers include VITON® (DuPont Dow Elastomers, Wilmington, Delaware, United States of America) and Kel-F type polymers, as described for microfluidic applications in U. S. Patent No. 6,408,878 to Unger et al. These commercially available polymers, however, have Mooney viscosities ranging from about 40 to 65 (ML 1+10 at 121°C) giving them a tacky, gum-like viscosity. When cured, they become a stiff, opaque solid. As currently available, VITON® and Kel-F have limited utility for micro-scale molding. Curable species of similar compositions, but having lower viscosity and greater optical clarity, is needed in the art for the applications described herein. A lower viscosity (e.g., 2 to 32 (ML 1+10 at 121°C)) or more preferably as low as 80 to 2000 cSt at 20 °C, composition yields a pourable liquid with a more efficient cure.

More particularly, the fluorine-containing olefins include, but are not limited to, vinylidine fluoride, hexafluoropropylene (HFP), tetrafluoroethylene (TFE), 1,2,3,3,3-pentafluoropropene (1-HPFP), chlorotrifluoroethylene (CTFE) and vinyl fluoride.

The fluorine-containing vinyl ethers include, but are not limited to perfluoro(alkyl vinyl) ethers (PAVEs). More particularly, perfluoro(alkyl vinyl) ethers for use as monomers include perfluoro(alkyl vinyl) ethers of the following formula:

$$CF_2=CFO(R_fO)_n(R_fO)_mR_f$$

wherein each R_f is independently a linear or branched C_1-C_6 perfluoroalkylene group, and m and n are each independently an integer from 0 to 10.

In some embodiments, the perfluoro(alkyl vinyl) ether comprises a monomer of the following formula:

$$CF_2=CFO(CF_2CFXO)_nR_f$$

wherein X is F or CF_3, n is an integer from 0 to 5, and R_f is a linear or branched C_1-C_6 perfluoroalkylene group. In some embodiments, n is 0 or 1 and R_f comprises 1 to 3 carbon atoms. Representative examples of such perfluoro(alkyl vinyl) ethers include perfluoro(methyl vinyl) ether (PMVE) and perfluoro(propyl vinyl) ether (PPVE).

In some embodiments, the perfluoro(alkyl vinyl) ether comprises a monomer of the following formula:

$$CF_2=CFO[(CF_2)_mCF_2CFZO)_nR_f$$

wherein R_f is a perfluoroalkyl group having 1-6 carbon atoms, m is an integer from 0 or 1, n is an integer from 0 to 5, and Z is F or CF_3. In some embodiments, R_f is C_3F_7, m is 0, and n is 1.

In some embodiments, the perfluoro(alkyl vinyl) ether monomers include compounds of the formula:

$$CF_2=CFO[(CF_2CF\{CF_3\}O)_n(CF_2CF_2CF_2O)_m(CF2)_p]C_xF_{2x+1}$$

wherein m and n each integers independently from 0 to 10, p is an integer from 0 to 3, and x is an integer from 1 to 5. In some embodiments, n is 0 or 1, m is 0 or 1, and x is 1.

Other examples of useful perfluoro(alkyl vinyl ethers) include:

$$CF_2=CFOCF_2CF(CF_3)O(CF_2O)_mC_nF_{2n+1}$$

wherein n is an integer from 1 to 5, m is an integer from 1 to 3. In some embodiments, n is 1.

In embodiments wherein copolymerized units of a perfluoro(alkyl vinyl) ether (PAVE) are present in the presently described fluoroelastomers, the PAVE content generally ranges from 25 to 75 weight percent, based on the total weight of the fluoroelastomer. If the PAVE is perfluoro(methyl vinyl) ether (PMVE), then the fluoroelastomer contains between 30 and 55 wt. % copolymerized PMVE units.

Hydrocarbon olefins useful in the presently described fluoroelastomers include, but are not limited to ethylene (E) and propylene (P). In embodiments wherein copolymerized units of a hydrocarbon olefin are present in the presently described fluoroelastomers, the hydrocarbon olefin content is generally 4 to 30 weight percent.

Further, the presently described fluoroelastomers can, in some embodiments, comprise units of one or more cure site monomers. Examples of suitable cure site monomers include: i) bromine -containing olefins; ii) iodine-containing olefins; iii) bromine-containing vinyl ethers; iv) iodine-containing vinyl ethers; v) fluorine-containing olefins having a nitrile group; vi) fluorine-containing vinyl ethers having a nitrile group; vii) 1,1,3,3,3-pentafluoropropene (2-HPFP); viii) perfluoro(2-phenoxypropyl vinyl) ether; and ix) non-conjugated dienes.

The brominated cure site monomers can contain other halogens, preferably fluorine. Examples of brominated olefin cure site monomers are $CF_2=CFOCF_2CF_2CF_2OCF_2CF_2Br$; bromotrifluoroethylene; 4-bromo-3,3,4,4-

tetrafluorobutene-1 (BTFB); and others such as vinyl bromide, 1-bromo-2,2-difluoroethylene; perfluoroallyl bromide; 4-bromo-1,1,2-trifluorobutene-1; 4-bromo-1,1,3,3,4,4,-hexafluorobutene; 4-bromo-3-chloro-1,1,3,4,4-pentafluorobutene; 6-bromo-5,5,6,6-tetrafluorohexene; 4-bromoperfluorobutene-1 and 3,3-difluoroallyl bromide. Brominated vinyl ether cure site monomers include 2-bromo-perfluoroethyl perfluorovinyl ether and fluorinated compounds of the class $CF_2Br\text{-}R_f\text{–}O\text{–}CF=CF_2$ (wherein R_f is a perfluoroalkylene group), such as $CF_2BrCF_2O\text{–}CF=CF_2$, and fluorovinyl ethers of the class ROCF=CFBr or $ROCBr=CF_2$ (wherein R is a lower alkyl group or fluoroalkyl group), such as $CH_3OCF=CFBr$ or $CF_3CH_2OCF=CFBr$.

Suitable iodinated cure site monomers include iodinated olefins of the formula: $CHR=CH\text{–}Z\text{–}CH_2CHR\text{–}I$, wherein R is –H or $–CH_3$; Z is a C_1 to C_{18} (per)fluoroalkylene radical, linear or branched, optionally containing one or more ether oxygen atoms, or a (per)fluoropolyoxyalkylene radical as disclosed in U.S. Pat. No. 5,674,959. Other examples of useful iodinated cure site monomers are unsaturated ethers of the formula: $I(CH_2CF_2CF_2)_nOCF=CF_2$ and $ICH_2CF_2\ O[CF(CF_3)CF_2O]_nCF=CF_2$, and the like, wherein n is an integer from 1 to 3, such as disclosed in U.S. Pat. No. 5,717,036. In addition, suitable iodinated cure site monomers including iodoethylene, 4-iodo-3,3,4,4-tetrafluorobutene-1 (ITFB); 3-chloro-4-iodo-3,4,4-trifluorobutene; 2-iodo-1,1,2,2-tetrafluoro-1-(vinyloxy)ethane; 2-iodo-1-(perfluorovinyloxy)-1,1,-2,2-tetrafluoroethylene; 1,1,2,3,3,3-hexafluoro-2-iodo-1-(perfluorovinyloxy)propane; 2-iodoethyl vinyl ether; 3,3,4,5,5,5-hexafluoro-4-iodopentene; and iodotrifluoroethylene are disclosed in U.S. Pat. No. 4,694,045. Allyl iodide and 2-iodo-perfluoroethyl perfluorovinyl ether also are useful cure site monomers.

Useful nitrile-containing cure site monomers include those of the formulas shown below:

$$CF_2=CF\text{–}O(CF_2)_n\text{–}CN$$

wherein n is an integer from 2 to 12. In some embodiments, n is an integer from 2 to 6.

$$CF_2=CF–O[CF_2-CF(CF)–O]_n–CF_2–CF(CF_3)–CN$$

wherein n is an integer from 0 to 4. In some embodiments, n is an integer from 0 to 2.

$$CF_2=CF–[OCF_2CF(CF_3)]_x–O–(CF_2)_n–CN$$

wherein x is 1 or 2, and n is an integer from 1 to 4; and

$$CF_2=CF–O–(CF_2)_n–O–CF(CF_3)–CN$$

wherein n is an integer from 2 to 4. In some embodiments, the cure site monomers are perfluorinated polyethers having a nitrile group and a trifluorovinyl ether group.

In some embodiments, the cure site monomer is:

$$CF_2=CFOCF_2CF(CF_3)OCF_2CF_2CN$$

i.e., perfluoro(8-cyano-5-methyl-3,6-dioxa-1-octene) or 8-CNVE.

Examples of non-conjugated diene cure site monomers include, but are not limited to 1,4-pentadiene; 1,5-hexadiene; 1,7-octadiene; 3,3,4,4-tetrafluoro-1,5-hexadiene; and others, such as those disclosed in Canadian Patent No. 2,067,891 and European Patent No. 0784064A1. A suitable triene is 8-methyl-4-ethylidene-1,7-octadiene.

In embodiments wherein the fluoroelastomer will be cured with peroxide, the cure site monomer is preferably selected from the group consisting of 4-bromo-3,3,4,4-tetrafluorobutene-1 (BTFB); 4-iodo-3,3,4,4-tetrafluorobutene-1 (ITFB); allyl iodide; bromotrifluoroethylene and 8-CNVE. In embodiments wherein the fluoroelastomer will be cured with a polyol, 2-HPFP or perfluoro(2-phenoxypropyl vinyl) ether is the preferred cure site monomer. In embodiments wherein the fluoroelastomer will be cured with a tetraamine, bis(aminophenol) or bis(thioaminophenol), 8-CNVE is the preferred cure site monomer.

Units of cure site monomer, when present in the presently disclosed fluoroelastomers, are typically present at a level of 0.05-10 wt. % (based on the total weight of fluoroelastomer), preferably 0.05-5 wt. % and most preferably between 0.05 and 3 wt. %.

Fluoroelastomers which can be used in the presently disclosed subject matter include, but are not limited to, those having at least 58 wt. % fluorine and comprising copolymerized units of i) vinylidene fluoride and hexafluoropropylene; ii) vinylidene fluoride, hexafluoropropylene and tetrafluoroethylene; iii) vinylidene fluoride, hexafluoropropylene, tetrafluoroethylene and 4-bromo-3,3,4,4-tetrafluorobutene-1; iv) vinylidene fluoride, hexafluoropropylene, tetrafluoroethylene and 4-iodo-3,3,4,4-tetrafluorobutene-1; v) vinylidene fluoride, perfluoro(methyl vinyl) ether, tetrafluoroethylene and 4-bromo-3,3,4,4-tetrafluorobutene-1; vi) vinylidene fluoride, perfluoro(methyl vinyl) ether, tetrafluoroethylene and 4-iodo-3,3,4,4-tetrafluorobutene-1; vii) vinylidene fluoride, perfluoro(methyl vinyl) ether, tetrafluoroethylene and 1,1,3,3,3-pentafluoropropene; viii) tetrafluoroethylene, perfluoro(methyl vinyl) ether and ethylene; ix) tetrafluoroethylene, perfluoro(methyl vinyl) ether, ethylene and 4-bromo-3,3,4,4-tetrafluorobutene-1; x) tetrafluoroethylene, perfluoro(methyl vinyl) ether, ethylene and 4-iodo-3,3,4,4-tetrafluorobutene-1; xi) tetrafluoroethylene, propylene and vinylidene fluoride; xii) tetrafluoroethylene and perfluoro(methyl vinyl) ether; xiii) tetrafluoroethylene, perfluoro(methyl vinyl) ether and perfluoro(8-cyano-5-methyl-3,6-dioxa-1-octene); xiv) tetrafluoroethylene, perfluoro(methyl vinyl) ether and 4-bromo-3,3,4,4-tetrafluorobutene-1; xv) tetrafluoroethylene, perfluoro(methyl vinyl) ether and 4-iodo-3,3,4,4-tetrafluorobutene-1; and xvi) tetrafluoroethylene, perfluoro(methyl vinyl) ether and perfluoro(2-phenoxypropyl vinyl) ether.

Additionally, iodine-containing endgroups, bromine-containing endgroups or combinations thereof can optionally be present at one or both of the fluoroelastomer polymer chain ends as a result of the use of chain transfer or molecular weight regulating agents during preparation of the fluoroelastomers. The amount of chain transfer agent, when employed, is calculated to result in an iodine or bromine level in the fluoroelastomer in the

range of 0.005-5 wt. %, preferably 0.05-3 wt. %.

Examples of chain transfer agents include iodine-containing compounds that result in incorporation of bound iodine at one or both ends of the polymer molecules. Methylene iodide; 1,4-diiodoperfluoro-n-butane; and 1,6-diiodo-3,3,4,4-tetrafluorohexane are representative of such agents. Other iodinated chain transfer agents include 1,3-diiodoperfluoropropane; 1,6-diiodoperfluorohexane; 1,3-diiodo-2-chloroperfluoropropane; 1,2-di(iododifluoromethyl)perfluorocyclobutane; monoiodoperfluoroethane; monoiodoperfluorobutane; 2-iodo-1-hydroperfluoroethane, and the like. Also included are the cyano-iodine chain transfer agents disclosed European Patent No. 0868447A1. Particularly preferred are diiodinated chain transfer agents.

Examples of brominated chain transfer agents include 1-bromo-2-iodoperfluoroethane; 1-bromo-3-iodoperfluoropropane; 1-iodo-2-bromo-1,1-difluoroethane and others such as disclosed in U.S. Patent No. 5,151,492.

Other chain transfer agents suitable for use include those disclosed in U.S. Patent No. 3,707,529. Examples of such agents include isopropanol, diethylmalonate, ethyl acetate, carbon tetrachloride, acetone and dodecyl mercaptan.

III. Method for Forming a Microfluidic Device Through a Thermal Free Radical Curing Process

In some embodiments, the presently disclosed subject matter provides a method for forming a microfluidic device by which a functional liquid perfluoropolyether (PFPE) precursor material is contacted with a patterned substrate, i.e., a master, and is thermally cured using a free radical initiator. As provided in more detail herein below, in some embodiments, the liquid PFPE precursor material is fully cured to form a fully cured PFPE network, which can then be removed from the patterned substrate and contacted with a second substrate to form a reversible, hermetic seal.

In some embodiments, the liquid PFPE precursor material is partially cured to form a partially cured PFPE network. In some embodiments, the partially cured network is contacted with a second partially cured layer of

PFPE material and the curing reaction is taken to completion, thereby forming a permanent bond between the PFPE layers.

Further, the partially cured PFPE network can be contacted with a layer or substrate comprising another polymeric material, such as poly(dimethylsiloxane) or another polymer, and then thermally cured so that the PFPE network adheres to the other polymeric material. Additionally, the partially cured PFPE network can be contacted with a solid substrate, such as glass, quartz, or silicon, and then bonded to the substrate through the use of a silane coupling agent.

III.A. Method of Forming a Patterned Layer of an Elastomeric Material

In some embodiments, the presently disclosed subject matter provides a method of forming a patterned layer of an elastomeric material. The presently disclosed method is suitable for use with the perfluoropolyether material described in Sections II.A. and II.B., and the fluoroolefin-based materials described in Section II.C. An advantage of using a higher viscosity PFPE material allows, among other things, for a higher molecular weight between cross links. A higher molecular weight between cross links can improve the elastomeric properties of the material, which can prevent among other things, cracks from forming. Referring now to Figures 1A-1C, a schematic representation of an embodiment of the presently disclosed subject matter is shown. A substrate **100** having a patterned surface **102** comprising a raised protrusion **104** is depicted. Accordingly, the patterned surface **102** of the substrate **100** comprises at least one raised protrusion **104**, which forms the shape of a pattern. In some embodiments, patterned surface **102** of substrate **100** comprises a plurality of raised protrusions **104** which form a complex pattern.

As best seen in Figure 1B, a liquid precursor material **106** is disposed on patterned surface **102** of substrate **100**. As shown in Figure 1B, the liquid precursor material **102** is treated with a treating process $\mathbf{T_r}$. Upon the treating of liquid precursor material **106**, a patterned layer **108** of an elastomeric material (as shown in Figure 1C) is formed.

As shown in Figure 1C, the patterned layer **108** of the elastomeric

material comprises a recess **110** that is formed in the bottom surface of the patterned layer **108**. The dimensions of recess **110** correspond to the dimensions of the raised protrusion **104** of patterned surface **102** of substrate **100**. In some embodiments, recess **110** comprises at least one channel **112**, which in some embodiments of the presently disclosed subject matter comprises a microscale channel. Patterned layer **108** is removed from patterned surface **102** of substrate **100** to yield microfluidic device **114**.

In some embodiments, the patterned substrate comprises an etched silicon wafer. In some embodiments, the patterned substrate comprises a photoresist patterned substrate. For the purposes of the presently disclosed subject matter, the patterned substrate can be fabricated by any of the processing methods known in the art, including, but not limited to, photolithography, electron beam lithography, and ion milling.

In some embodiments, the patterned layer of perfluoropolyether is between about 0.1 micrometers and about 100 micrometers thick. In some embodiments, the patterned layer of perfluoropolyether is between about 0.1 millimeters and about 10 millimeters thick. In some embodiments, the patterned layer of perfluoropolyether is between about one micrometer and about 50 micrometers thick. In some embodiments, the patterned layer of perfluoropolyether is about 20 micrometers thick. In some embodiments, the patterned layer of perfluoropolyether is about 5 millimeters thick.

In some embodiments, the patterned layer of perfluoropolyether comprises a plurality of microscale channels. In some embodiments, the channels have a width ranging from about 0.01 μm to about 1000 μm; a width ranging from about 0.05 μm to about 1000 μm; and/or a width ranging from about 1 μm to about 1000 μm. In some embodiments, the channels have a width ranging from about 1 μm to about 500 μm; a width ranging from about 1 μm to about 250 μm; and/or a width ranging from about 10 μm to about 200 μm. Exemplary channel widths include, but are not limited to, 0.1 μm, 1 μm, 2 μm, 5 μm, 10 μm, 20 μm, 30 μm, 40 μm, 50 μm, 60 μm, 70 μm, 80 μm, 90 μm, 100 μm, 110 μm, 120 μm, 130 μm, 140 μm, 150 μm, 160 μm, 170 μm, 180 μm, 190 μm, 200 μm, 210 μm, 220 μm, 230 μm, 240 μm, and 250 μm.

In some embodiments, the channels have a depth ranging from about

1 μm to about 1000 μm; and/or a depth ranging from about 1 μm to 100 μm. In some embodiments, the channels have a depth ranging from about 0.01 μm to about 1000 μm; a depth ranging from about 0.05 μm to about 500 μm; a depth ranging from about 0.2 μm to about 250 μm; a depth ranging from about 1 μm to about 100 μm; a depth ranging from about 2 μm to about 20 μm; and/or a depth ranging from about 5 μm to about 10 μm. Exemplary channel depths include, but are not limited to, 0.01 μm, 0.02 μm, 0.05 μm, 0.1 μm, 0.2 μm, 0.5 μm, 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 7.5 μm, 10 μm, 12.5 μm, 15 μm, 17.5 μm, 20 μm, 22.5 μm, 25 μm, 30 μm, 40 μm, 50 μm, 75 μm, 100 μm, 150 μm, 200 μm, and 250 μm.

In some embodiments, the channels have a width-to-depth ratio ranging from about 0.1:1 to about 100:1. In some embodiments, the channels have a width-to-depth ratio ranging from about 1:1 to about 50:1. In some embodiments, the channels have a width-to-depth ratio ranging from about 2:1 to about 20:1. In some embodiments, the channels have a width-to-depth ratio ranging from about 3:1 to about 15:1. In some embodiments, the channels have a width-to-depth ratio of about 10:1.

One of ordinary skill in the art would recognize that the dimensions of the channels of the presently disclosed subject matter are not limited to the exemplary ranges described hereinabove and can vary in width and depth to affect the magnitude of force required to flow a material through the channel and/or to actuate a valve to control the flow of the material therein. Further, as will be described in more detail herein below, channels of greater width are contemplated for use as a fluid reservoir, a reaction chamber, a mixing channel, a separation region, and the like.

III.B. Method for Forming a Multilayer Patterned Material

In some embodiments, the presently disclosed subject matter describes a method for forming a multilayer patterned material, e.g., a multilayer patterned PFPE material. In some embodiments, the multilayer patterned perfluoropolyether material is used to fabricate a monolithic PFPE-based microfluidic device.

Referring now to Figures 2A-2D, a schematic representation of the

preparation of an embodiment of the presently disclosed subject matter is shown. Patterned layers **200** and **202** are provided, each of which, in some embodiments, comprise a perfluoropolyether material prepared from a liquid PFPE precursor material having a viscosity greater than about 100 cSt. In this example, each of the patterned layers **200** and **202** comprise a plurality of channels **204**. In this embodiment of the presently disclosed subject matter, the plurality of channels **204** comprise microscale channels. In patterned layer **200**, the channels are represented by a dashed line, i.e., in shadow, in Figures 2A-2C. Patterned layer **202** is overlaid on patterned layer **200** in a predetermined alignment. In this example, the predetermined alignment is such that channels **204** in patterned layer **200** and **202** are substantially perpendicular to each other. In some embodiments, as depicted in Figures 2A-2D, patterned layer **200** is overlaid on nonpatterned layer **206**. Nonpatterned layer **206** can comprise a perfluoropolyether.

Continuing with reference to Figures 2A-2D, patterned layers **200** and **202**, and in some embodiments nonpatterned layer **206**, are treated by a treating process T_r. As described in more detail herein below, layers **200**, **202**, and, in some embodiments nonpatterned layer **206**, are treated by treating T_r, to promote the adhesion of patterned layers **200** and **202** to each other, and in some embodiments, patterned layer **200** to nonpatterned layer **206**, as shown in Figures 2C and 2D. The resulting microfluidic device **208** comprises an integrated network **210** of microscale channels **204** which intersect predetermined intersecting points **212**, as best seen in the cross-section provided in Figure 2D. Also shown in Figure 2D is membrane **214** comprising the top surface of channels **204** of patterned layer **200** which separates channel **204** of patterned layer **202** from channels **204** of patterned layer **200**.

Continuing with reference to Figures 2A-2C, in some embodiments, patterned layer **202** comprises a plurality of apertures, and the apertures are designated input aperture **216** and output aperture **218**. In some embodiments, the holes, e.g., input aperture **216** and output aperture **218** are in fluid communication with channels **204**. In some embodiments, the apertures comprise a side-actuated valve structure comprising a thin

membrane of PFPE material which can be actuated to restrict the flow in an abutting channel (not shown).

In some embodiments, the first patterned layer of photocured PFPE material is cast at such a thickness to impart a degree of mechanical stability to the PFPE structure. Accordingly, in some embodiments, the first patterned layer of the photocured PFPE material is about 50 μm to several centimeters thick. In some embodiments, the first patterned layer of the photocured PFPE material is between 50 μm and about 10 millimeters thick. In some embodiments, the first patterned layer of the photocured PFPE material is 5 mm thick. In some embodiments, the first patterned layer of PFPE material is about 4 mm thick. Further, in some embodiments, the thickness of the first patterned layer of PFPE material ranges from about 0.1 μm to about 10 cm; from about 1 μm to about 5 cm; from about 10 μm to about 2 cm; and from about 100 μm to about 10 mm.

In some embodiments, the second patterned layer of the photocured PFPE material is between about 1 micrometer and about 100 micrometers thick. In some embodiments, the second patterned layer of the photocured PFPE material is between about 1 micrometer and about 50 micrometers thick. In some embodiments, the second patterned layer of the photocured material is about 20 micrometers thick.

Although Figures 2A-2C disclose the formation of a microfluidic device wherein two patterned layers of PFPE material are combined, in some embodiments of the presently disclosed subject matter it is possible to form a microfluidic device comprising one patterned layer and one non-patterned layer of PFPE material. Thus, the first patterned layer can comprise a microscale channel or an integrated network of microscale channels and then the first patterned layer can be overlaid on top of the non-patterned layer and adhered to the non-patterned layer using a photocuring step, such as application of ultraviolet light as disclosed herein, to form a monolithic structure comprising enclosed channels therein.

Accordingly, in some embodiments, a first and a second patterned layer of photocured perfluoropolyether material, or alternatively a first patterned layer of photocured perfluoropolyether material and a nonpatterned

layer of photocured perfluoropolyether material, adhere to one another, thereby forming a monolithic PFPE-based microfluidic device.

III.C. Method of Forming a Patterned PFPE Layer Through a Thermal Free Radical Curing Process

In some embodiments, a thermal free radical initiator, including, but not limited to, a peroxide and/or an azo compound, is blended with a liquid perfluoropolyether (PFPE) precursor, which is functionalized with a polymerizable group, including, but not limited to, an acrylate, a methacrylate, and a styrenic unit to form a blend. As shown in Figures 1A-1C, the blend is then contacted with a patterned substrate, i.e., a "master," and heated to cure the PFPE precursor into a network.

In some embodiments, the PFPE precursor is fully cured to form a fully cured PFPE precursor. In some embodiments, the free radical curing reaction is allowed to proceed only partially to form a partially-cured network.

III.D. Method of Adhering a Layer of a PFPE Material to a Substrate Through a Thermal Free Radical Curing Process

In some embodiments the fully cured PFPE precursor is removed, e.g., peeled, from the patterned substrate, i.e., the master, and contacted with a second substrate to form a reversible, hermetic seal.

In some embodiments, the partially cured network is contacted with a second partially cured layer of PFPE material and the curing reaction is taken to completion, thereby forming a permanent bond between the PFPE layers.

In some embodiments, the partial free-radical curing method is used to bond at least one layer of a partially-cured PFPE material to a substrate. In some embodiments, the partial free-radical curing method is used to bond a plurality of layers of a partially-cured PFPE material to a substrate. In some embodiments, the substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. In some embodiments, the substrate is treated with a silane coupling agent.

An embodiment of the presently disclosed method for adhering a layer

of PFPE material to a substrate is illustrated in Figures 3A-3C. Referring now to Figure 3A, a substrate **300** is provided, wherein, in some embodiments, substrate **300** is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. Substrate **300** is treated by treating process $\mathbf{T_{r1}}$. In some embodiments, treating process $\mathbf{T_{r1}}$ comprises treating the substrate with a base/alcohol mixture, e.g., KOH/isopropanol, to impart a hydroxyl functionality to substrate **300**.

Referring now to Figure 3B, functionalized substrate **300** is reacted with a silane coupling agent, e.g., R-$SiCl_3$ or R-$Si(OR_1)_3$, wherein R and R_1 represent a functional group as described herein to form a silanized substrate **300**. In some embodiments, the silane coupling agent is selected from the group consisting of a monohalosilane, a dihalosilane, a trihalosilane, a monoalkoxysilane, a dialkoxysilane, and a trialkoxysilane; and wherein the monohalosilane, dihalosilane, trihalosilane, monoalkoxysilane, dialkoxysilane, and trialkoxysilane are functionalized with a moieties selected from the group consisting of an amine, a methacrylate, an acrylate, a styrenic, an epoxy, an isocyanate, a halogen, an alcohol, a benzophenone derivative, a maleimide, a carboxylic acid, an ester, an acid chloride, and an olefin.

Referring now to Figure 3C, silanized substrate **300** is contacted with a patterned layer of partially cured PFPE material **302** and treated by treating process $\mathbf{Tr_2}$ to form a permanent bond between patterned layer of PFPE material **302** and substrate **300**.

In some embodiments, a partial free radical cure is used to adhere a PFPE layer to a second polymeric material, such as a poly(dimethyl siloxane) (PDMS) material, a polyurethane material, a silicone-containing polyurethane material, and a PFPE-PDMS block copolymer material. In some embodiments, the second polymeric material comprises a functionalized polymeric material. In some embodiments, the second polymeric material is encapped with a polymerizable group. In some embodiments, the polymerizable group is selected from the group consisting of an acrylate, a styrene, and a methacrylate. Further, in some embodiments, the second polymeric material is treated with a plasma and a silane coupling agent to

introduce the desired functionality to the second polymeric material.

An embodiment of the presently disclosed method for adhering a patterned layer of PFPE material to another patterned layer of polymeric material is illustrated in Figures 4A-4C. Referring now to Figure 4A, a patterned layer of a first polymeric material **400** is provided. In some embodiments, first polymeric material comprises a PFPE material. In some embodiments, first polymeric material comprises a polymeric material selected from the group consisting of a poly(dimethylsiloxane) material, a polyurethane material, a silicone-containing polyurethane material, and a PFPE-PDMS block copolymer material. Patterned layer of first polymeric material **400** is treated by treating process $\mathbf{T_{r1}}$. In some embodiments, treating process $\mathbf{T_{r1}}$ comprises exposing the patterned layer of first polymeric material **400** to UV light in the presence of O_3 and an R functional group, to add an R functional group to the patterned layer of polymeric material **400**.

Referring now to Figure 4B, the functionalized patterned layer of first polymeric material **400** is contacted with the top surface of a functionalized patterned layer of PFPE material **402** and then treated by treating process $\mathbf{T_{r2}}$ to form a two layer hybrid assembly **404**. Thus, functionalized patterned layer of first polymeric material **400** is thereby bonded to functionalized patterned layer of PFPE material **402**.

Referring now to Figure 4C, two-layer hybrid assembly **404**, in some embodiments, is contacted with substrate **406** to form a multilayer hybrid structure **410**. In some embodiments, substrate **406** is coated with a layer of liquid PFPE precursor material **408**. Multilayer hybrid structure **410** is treated by treating process $\mathbf{T_{r3}}$ to bond two-layer assembly **404** to substrate **406**.

IV. Method for Forming a Microfluidic Device Through a Two-Component Curing Process

The presently disclosed subject matter provides a method for forming a microfluidic device by which functional perfluoropolyether (PFPE) precursors are contacted with a patterned surface and then cured through the reaction of two components, such as epoxy/amine, hydroxyl/isocyanate, hydroxyl/acid chloride, and hydroxyl/chlorosilane, to form a fully-cured or a partially-cured

PFPE network. In some embodiments, the partially-cured PFPE network is contacted with another substrate, and the curing is then take to completion to adhere the PFPE network to the substrate. This method can be used to adhere multiple layers of a PFPE material to a substrate.

Further, in some embodiments, the substrate comprises a second polymeric material, such as PDMS, or another polymer. In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic material, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

In some embodiments, the PFPE layer is adhered to a solid substrate, such as a glass material, a quartz material, a silicon material, and a fused silica material, through use of a silane coupling agent.

IV. A. Method of Forming a Patterned PFPE Layer Through a Two-Component Curing Process

In some embodiments, a PFPE network is formed through the reaction of a two-component functional liquid precursor system. Using the general method for forming a patterned layer of polymeric material as shown in Figures 1A-1C, a liquid precursor material comprising a two-component system is contacted with a patterned substrate and a patterned layer of PFPE material is formed. In some embodiments, the two-component liquid precursor system is selected from the group consisting of an epoxy/amine system, a hydroxyl/isocyanate system, an amine/isocyanate system, a hydroxyl/acid chloride system, and a hydroxyl/chlorosilane system. The functional liquid precursors are blended in the appropriate ratios and then contacted with a patterned surface or master. The curing reaction is allowed to take place by using heat, catalysts, and the like, until the network is formed.

In some embodiments, a fully cured PFPE precursor is formed. In

some embodiments, the two-component reaction is allowed to proceed only partially, thereby forming a partially cured PFPE network.

IV. B. Method of Adhering a PFPE Layer to a Substrate Through a Two-Component Curing Process

IV.B.1. Full Cure with a Two-Component Curing Process

In some embodiments, the fully cured PFPE two-component precursor is removed, e.g., peeled, from the master and contacted with a substrate to form a reversible, hermetic seal. In some embodiments, the partially cured network is contacted with another partially cured layer of PFPE and the reaction is taken to completion, thereby forming a permanent bond between the layers.

IV.B.2. Partial Cure with a Two-Component System

As shown in Figures 3A-3C, in some embodiments, the partial two-component curing method is used to bond at least one layer of a partially-cured PFPE material to a substrate. In some embodiments, the partial two-component curing method is used to bond a plurality of layers of a partially-cured PFPE material to a substrate. In some embodiments, the substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. In some embodiments, the substrate is treated with a silane coupling agent.

As shown in Figures 4A-4C, in some embodiments, a partial two-component cure is used to adhere the PFPE layer to a second polymeric material, such as a poly(dimethylsiloxane) (PDMS) material. In some embodiments, the PDMS material comprises a functionalized PDMS material. In some embodiments, the PDMS is treated with a plasma and a silane coupling agent to introduce the desired functionality to the PDMS material. In some embodiments, the PDMS material is encapped with a polymerizable group. In some embodiments, the polymerizable group comprises an epoxide. In some embodiments, the polymerizable group comprises an amine.

In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

IV.B.3. Excess Cure with a Two-Component System

The presently disclosed subject matter provides a method for forming a microfluidic device by which a functional perfluoropolyether (PFPE) precursor is contacted with a patterned substrate and cured through the reaction of two components, such as epoxy/amine, hydroxyl/isocyanate, hydroxyl/acid chloride, and hydroxyl/chlorosilane, to form a layer of cured PFPE material. In this particular method, the layer of cured PFPE material can be adhered to a second substrate by fully curing the layer with an excess of one component and contacting the layer of cured PFPE material with a second substrate comprising an excess of a second component in such a way that the excess groups react to adhere the layers.

Thus, in some embodiments, a two-component system, such as an epoxy/amine system, a hydroxyl/isocyanate system, an amine/isocyanate system, a hydroxyl/acid chloride system, or a hydroxyl/chlorosilane system, is blended. In some embodiments, at least one component of the two-component system is in excess of the other component. The reaction is then taken to completion by heating, using a catalyst, and the like, with the remaining cured network comprising a plurality of functional groups generated by the presence of the excess component.

In some embodiments, two layers of fully cured PFPE materials comprising complimentary excess groups are contacted with one another, wherein the excess groups are allowed to react, thereby forming a permanent bond between the layers.

As shown in Figures 3A-3C, in some embodiments, a fully cured PFPE network comprising excess functional groups is contacted with a substrate. In some embodiments, the substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. In some embodiments, the substrate is treated with a silane coupling agent such that the functionality on the coupling agent is complimentary to the excess functionality on the fully cured network. Thus, a permanent bond is formed to the substrate.

As shown in Figures 4A-4C, in some embodiments, the two-component excess cure is used to bond a PFPE network to a second polymeric material, such as a poly(dimethylsiloxane) PDMS material. In some embodiments, the PDMS material comprises a functionalized PDMS material. In some embodiments, the PDMS material is treated with a plasma and a silane coupling agent to introduce the desired functionality. In some embodiments, the PDMS material is encapped with a polymerizable group. In some embodiments, the polymerizable material comprises an epoxide. In some embodiments, the polymerizable material comprises an amine.

In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

V. Method for Functionalizing a Surface of a Micro- and/or Nano-scale Device

In some embodiments, the presently disclosed subject matter provides materials and methods for functionalizing the channels in a microfluidic device and/or a microtiter well. In some embodiments, such functionalization includes, but is not limited to, the synthesis and/or attachment of peptides and

other natural polymers to the interior surface of a channel in a microfluidic device. Accordingly, the presently disclosed subject matter can be applied to microfluidic devices, such as those described by Rolland, J., et al., *JACS* **2004**, *126,* 2322-2323, the disclosure of which is incorporated herein by reference in its entirety.

In some embodiments, the method comprises binding a small molecule to the interior surface of a microfluidic channel or the surface of a microtiter well. In such embodiments, once bound, the small molecule can serve a variety of functions. In some embodiments, the small molecule functions as a cleavable group, which when activated, can change the polarity of the channel and hence the wettability of the channel. In some embodiments, the small molecule functions as a binding site. In some embodiments, the small molecule functions as a binding site for one of a catalyst, a drug, a substrate for a drug, an analyte, and a sensor. In some embodiments, the small molecule functions as a reactive functional group. In some embodiments, the reactive functional group is reacted to yield a Zwitterion. In some embodiments, the Zwitterion provides a polar, ionic channel.

An embodiment of the presently disclosed method for functionalizing the interior surface of a microfluidic channel and/or a microtiter well is illustrated in Figures 5A and 5B. Referring now to Figure 5A, a microfluidic channel **500** is provided. In some embodiments, microfluidic channel **500** is formed from a functional PFPE material comprising an **R** functional group, as described herein. In some embodiments, microchannel **500** comprises a PFPE network which undergoes a post-curing treating process, whereby functional group **R** is introduced into the interior surface **502** of microfluidic channel **500**.

Referring now to Figure 5B, a microtiter well **504** is provided. In some embodiments, microtiter well **504** is coated with a layer of functionalized PFPE material **506**, which comprises an **R** functional group, to impart functionality into microtiter well **504**.

V.A. Method of Attaching a Functional Group to a PFPE Network

In some embodiments, PFPE networks comprising excess functionality are used to functionalize the interior surface of a microfluidic channel or the surface of a microtiter well. In some embodiments, the interior surface of a microfluidic channel or the surface of a microtiter well is functionalized by attaching a functional moiety selected from the group consisting of a protein, an oligonucleotide, a drug, a ligand, a catalyst, a dye, a sensor, an analyte, and a charged species capable of changing the wettability of the channel.

In some embodiments, latent functionalities are introduced into the fully cured PFPE network. In some embodiments, latent methacrylate groups are present at the surface of the PFPE network that has been free radically cured either photochemically or thermally. Multiple layers of fully cured PFPE are then contacted with the functionalized surface of the PFPE network, forming a seal, and reacted, by heat, for example, to allow the latent functionalities to react and form a permanent bond between the layers.

In some embodiments, the latent functional groups react photochemically with one another at a wavelength different from that used to cured PFPE precursors. In some embodiments, this method is used to adhere fully cured layers to a substrate. In some embodiments, the substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. In some embodiments, the substrate is treated with a silane coupling agent complimentary to the latent functional groups.

In some embodiments, such latent functionalities are used to adhere a fully cured PFPE network to a second polymeric material, such as a poly(dimethylsiloxane) PDMS material. In some embodiments, the PDMS material comprises a functionalized PDMS material. In some embodiments, the PDMS material is treated with a plasma and a silane coupling agent to introduce the desired functionality. In some embodiments, the PDMS material is encapped with a polymerizable group. In some embodiments, the polymerizable group is selected from the group consisting of an acrylate, a styrene, and a methacrylate.

In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

V.B. Method of Introducing Functionality in the Generation of a Liquid PFPE Precursor

The presently disclosed subject matter provides a method of forming a microfluidic device by which a photochemically cured PFPE layer is placed in conformal contact with a second substrate thereby forming a seal. The PFPE layer is then heated at elevated temperatures to adhere the layer to the substrate through latent functional groups. In some embodiments, the second substrate also comprises a cured PFPE layer. In some embodiments, the second substrate comprises a second polymeric material, such as a poly(dimethylsiloxane) (PDMS) material.

In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

In some embodiments, the latent groups comprise methacrylate units that are not reacted during the photocuring process. Further, in some embodiments, the latent groups are introduced in the generation of the liquid PFPE precursor. For example, in some embodiments, methacrylate units are

added to a PFPE diol through the use of glycidyl methacrylate, the reaction of the hydroxy and the epoxy group generates a secondary alcohol, which can be used as a handle to introduce chemical functionality. In some embodiments, multiple layers of fully cured PFPE are adhered to one another through these latent functional groups. In some embodiments, the latent functionalities are used to adhere a fully cured PFPE layer to a substrate. In some embodiments, the substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. In some embodiments, the substrate is treated with a silane coupling agent.

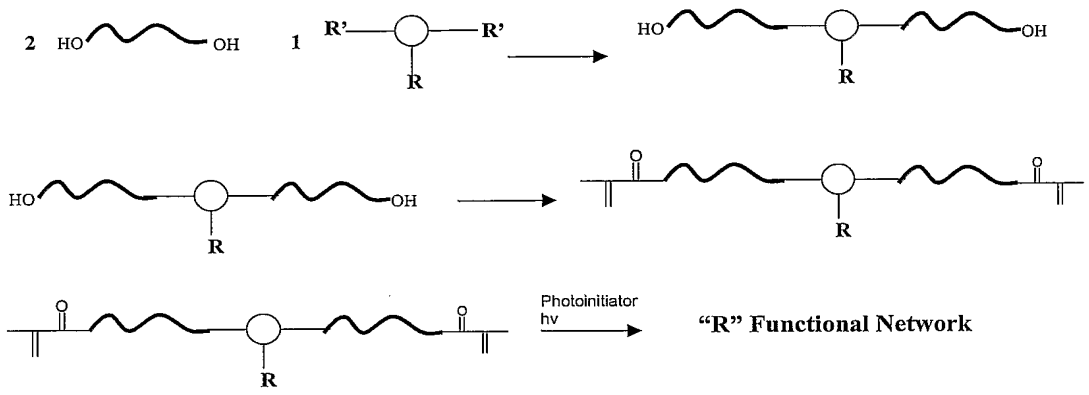
Further, this method can be used to adhere a fully cured PFPE layer to a second polymeric material, such as a poly(dimethylsiloxane) (PDMS) material. In some embodiments, the PDMS material comprises a functionalized PDMS material. In some embodiments, the PDMS material is treated with a plasma and a silane coupling agent to introduce the desired functionality. In some embodiments, the PDMS material is encapped with a polymerizable group. In some embodiments, the polymerizable material is selected from the group consisting of an acrylate, a styrene, and a methacrylate.

In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

In some embodiments, PFPE networks containing latent functionality are used to functionalize the interior surface of a microfluidic channel or a microtiter well. Examples include the attachment of proteins, oligonucleotides, drugs, ligands, catalysts, dyes, sensors, analytes, and charged species capable of changing the wettability of the channel.

V.C. Method of Linking Multiple Chains of a PFPE Material with a Functional Linker Group

In some embodiments, the presently disclosed method adds functionality to a microfluidic channel or a microtiter well by adding a chemical "linker" moiety to the elastomer itself. In some embodiments, a functional group is added along the backbone of the precursor material. An example of this method is illustrated in Scheme 6.



Scheme 6. Representative method of adding a functional group along the backbone of a precursor material.

In some embodiments, the precursor material comprises a macromolecule containing hydroxyl functional groups. In some embodiments, as depicted in Scheme 6, the hydroxyl functional groups comprise diol functional groups. In some embodiments, two or more of the diol functional groups are connected through a trifunctional "linker" molecule. In some embodiments, the trifunctional linker molecule has two functional groups, R and R'. In some embodiments, the R' group reacts with the hydroxyl groups of the macromolecule. In Scheme 6, the circle can represent a linking molecule; and the wavy line can represent a PFPE chain.

In some embodiments, the R group provides the desired functionality to the interior surface of the microfluidic channel or surface of a microtiter well. In some embodiments, the R' group is selected from the group including, but not limited to, an acid chloride, an isocyanate, a halogen, and an ester moiety.

In some embodiments, the R group is selected from one of, but not limited to, a protected amine and a protected alcohol. In some embodiments, the macromolecule diol is functionalized with polymerizable methacrylate groups. In some embodiments, the functionalized macromolecule diol is cured and/or molded by a photochemical process as described by Rolland, J. et al. *JACS* **2004**, *126*, 2322-2323, the disclosure of which is incorporated herein by reference in its entirety.

Thus, the presently disclosed subject matter provides a method of incorporating latent functional groups into a photocurable PFPE material through a functional linker group. Thus, in some embodiments, multiple chains of a PFPE material are linked together before encapping the chain with a polymerizable group. In some embodiments, the polymerizable group is selected from the group consisting of a methacrylate, an acrylate, and a styrenic. In some embodiments, latent functionalities are attached chemically to such "linker" molecules in such a way that they will be present in the fully cured network.

In some embodiments, latent functionalities introduced in this manner are used to bond multiple layers of PFPE, bond a fully cured PFPE layer to a substrate, such as a glass material or a silicon material that has been treated with a silane coupling agent, or bond a fully cured PFPE layer to a second polymeric material, such as a PDMS material. In some embodiments, the PDMS material is treated with a plasma and a silane coupling agent to introduce the desired functionality. In some embodiments, the PDMS material is encapped with a polymerizable group. In some embodiments, the polymerizable group is selected from the group consisting of an acrylate, a styrene, and a methacrylate.

In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride,

a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

In some embodiments, PFPE networks comprising functionality attached to "linker" molecules are used to functionalize the interior surface of a microfluidic channel and/or the surface of a microtiter well. In some embodiments, the inside of a microfluidic channel is functionalized by attaching a functional moiety selected from the group consisting of a protein, an oligonucleotide, a drug, a catalyst, a dye, a sensor, an analyte, and a charged species capable of changing the wettability of the channel.

VI. Method of Adding Functional Monomers to the PFPE Precursor Material

In some embodiments, the method comprises adding a functional monomer to an uncured precursor material. In some embodiments, the functional monomer is selected from the group consisting of functional styrenes, methacrylates, and acrylates. In some embodiments, the precursor material comprises a fluoropolymer. In some embodiments, the functional monomer comprises a highly fluorinated monomer. In some embodiments, the highly fluorinated monomer comprises perfluoro ethyl vinyl ether (EVE). In some embodiments, the precursor material comprises a poly(dimethyl siloxane) (PDMS) elastomer. In some embodiments, the precursor material comprises a polyurethane elastomer. In some embodiments, the method further comprises incorporating the functional monomer into the network by a curing step.

In some embodiments, functional monomers are added directly to the liquid PFPE precursor to be incorporated into the network upon crosslinking. For example, monomers can be introduced into the network that are capable of reacting post-crosslinking to adhere multiple layers of PFPE, bond a fully cured PFPE layer to a substrate, such as a glass material or a silicon material that has been treated with a silane coupling agent, or bond a fully cured PFPE layer to a second polymeric material, such as a PDMS material. In some embodiments, the PDMS material is treated with a plasma and a silane coupling agent to introduce the desired functionality. In some embodiment,

the PDMS material is encapped with a polymerizable group. In some embodiments, the polymerizable material is selected from the group consisting of an acrylate, a styrene, and a methacrylate.

In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

In some embodiments, functional monomers are added directly to the liquid PFPE precursor and are used to attach a functional moiety selected from the group consisting of a protein, an oligonucleotide, a drug, a catalyst, a dye, a sensor, an analyte, and a charged species capable of changing the wettability of the channel.

Such monomers include, but are not limited to, tert-butyl methacrylate, tert butyl acrylate, dimethylaminopropyl methacrylate, glycidyl methacrylate, hydroxy ethyl methacrylate, aminopropyl methacrylate, allyl acrylate, cyano acrylates, cyano methacrylates, trimethoxysilane acrylates, trimethoxysilane methacrylates, isocyanato methacrylate, lactone-containing acrylates and methacrylates, sugar-containing acrylates and methacrylates, poly-ethylene glycol methacrylate, nornornane-containing methacrylates and acrylates, polyhedral oligomeric silsesquioxane methacrylate, 2-trimethylsiloxyethyl methacrylate, 1H,1H,2H,2H-fluoroctylmethacrylate, pentafluorostyrene, vinyl pyridine, bromostyrene, chlorostyrene, styrene sulfonic acid, fluorostyrene, styrene acetate, acrylamide, and acrylonitrile.

In some embodiments, monomers which already have the above agents attached are blended directly with the liquid PFPE precursor to be incorporated into the network upon crosslinking. In some embodiments, the monomer comprises a group selected from the group consisting of a polymerizable group, the desired agent, and a fluorinated segment to allow for

miscibility with the PFPE liquid precursor. In some embodiments, the monomer does not comprise a polymerizable group, the desired agent, and a fluorinated segment to allow for miscibility with the PFPE liquid precursor.

In some embodiments, monomers are added to adjust the mechanical properties of the fully cured elastomer. Such monomers include, but are not limited to: perfluoro(2,2-dimethyl-1,3-dioxole), hydrogen-bonding monomers which contain hydroxyl, urethane, urea, or other such moieties, monomers containing bulky side group, such as tert-butyl methacrylate.

In some embodiments, functional species such as the above mentioned monomers are introduced and are mechanically entangled, i.e., not covalently bonded, into the network upon curing. For example, in some embodiments, functionalities are introduced to a PFPE chain that does not contain a polymerizable monomer and such a monomer is blended with the curable PFPE species. In some embodiments, such entangled species can be used to adhere multiple layers of cured PFPE together if two species are reactive, such as: epoxy/amine, hydroxy/acid chloride, hydroxy/isocyanate, amine/isocyanate, amine/halide, hydroxy/halide, amine/ester, and amine/carboxylic acid. Upon heating, the functional groups will react and adhere the two layers together.

Additionally, such entangled species can be used to adhere a PFPE layer to a layer of another material, such as glass, silicon, quartz, PDMS, Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

In some embodiments, such an entangled species can be used to functionalize the interior of a microfluidic channel for the purposes described hereinabove.

VII. Other Methods of Introducing Functionality to a PFPE Surface

In some embodiments, an Argon plasma is used to introduce functionality along a fully cured PFPE surface using the method for functionalizing a poly(tetrafluoroethylene) surface as described by Chen, Y. and Momose, Y. *Surf. Interface. Anal.* **1999**, *27*, 1073-1083, which is incorporated herein by reference in it entirety. More particularly, without being bound to any one particular theory, exposure of a fully cured PFPE material to Argon plasma for a period of time adds functionality along the fluorinated backbone.

Such functionality can be used to adhere multiple layers of PFPE, bond a fully cured PFPE layer to a substrate, such as a glass material or a silicon material that has been treated with a silane coupling agent, or bond a fully cured PFPE layer to a second polymeric material, such as a PDMS material. In some embodiments, the PDMS material comprises a functionalized material. In some embodiments, the PDMS material is treated with a plasma and a silane coupling agent to introduce the desired functionality. Such functionalities also can be used to attach proteins, oligonucleotides, drugs, catalysts, dyes, sensors, analytes, and charged species capable of changing the wettability of the channel.

In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).

In some embodiments, a fully cured PFPE layer is brought into conformal contact with a solid substrate. In some embodiments, the solid substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. In some embodiments, the PFPE material is irradiated with UV light, e.g., a 185-

nm UV light, which can strip a fluorine atom off of the back bone and form a chemical bond to the substrate as described by Vurens, G., et al. *Langmuir* **1992,** *8*, 1165-1169. Thus, in some embodiments, the PFPE layer is covalently bonded to the solid substrate by radical coupling following abstraction of a fluorine atom.

VIII. Adhesion of a Microscale or a Nanoscale Device to a Substrate through an Encasing Polymer

In some embodiments, a microscale device, a nanoscale device, or combinations thereof is adhered to a substrate by placing the fully cured device in conformal contact on the substrate and pouring an "encasing polymer" over the entire device. In some embodiments, the encasing polymer is selected from the group consisting of a liquid epoxy precursor and a polyurethane. The encasing polymer is then solidified by curing or other methods. The encasement serves to bind the layers together mechanically and to bind the layers to the substrate.

In some embodiments, the microscale device, the nanoscale device, or combinations thereof comprises one of a perfluoropolyether material as described in Section II.A and Section II.B. hereinabove and a fluoroolefin-based material as described in Section II.C. hereinabove.

In some embodiments, the substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. Further, in some embodiments, the substrate comprises a second polymeric material, such as poly(dimethylsiloxane) (PDMS), or another polymer. In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic material, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone).In some

embodiments, the surface of the substrate is functionalized with a silane coupling agent such that it will react with the encasing polymer to form an irreversible bond.

IX. Method for Forming a Microstructure Using Sacrificial Layers

The presently disclosed subject matter provides a method for forming microchannels or a microstructure for use as a microfluidic device by using sacrificial layers comprising a degradable or selectively soluble material. In some embodiments, the method comprises contacting a liquid precursor material with a two-dimensional or a three-dimensional sacrificial structure, treating, e.g., curing, the precursor material, and removing the sacrificial structure to form a microfluidic channel.

Accordingly, in some embodiments, a PFPE liquid precursor is disposed on a multidimensional scaffold, wherein the multidimensional scaffold is fabricated from a material that can be degraded or washed away after curing of the PFPE network. These materials protect the channels from being filled in when another layer of elastomer is cast thereon. Examples of such degradable or selective soluble materials include, but are not limited to waxes, photoresists, polysulfones, polylactones, cellulose fibers, salts, or any solid organic or inorganic compounds. In some embodiments, the sacrificial layer is removed thermally, photochemically, or by washing with solvents. Importantly, the compatibility of the materials and devices disclosed herein with organic solvents provides the capability to use sacrificial polymer structures in microfluidic devices.

The PFPE materials of use in forming a microstructure by using sacrificial layers include those PFPE and fluoroolefin-based materials as described hereinabove in Section II of the presently disclosed subject matter.

Figures 6A-6D and Figures 7A-7C show embodiments of the presently disclosed methods for forming a microstructure by using a sacrificial layer of a degradable or selectively soluble material.

Referring now to Figure 6A, a patterned substrate **600** is provided. Liquid PFPE precursor material **602** is disposed on patterned substrate **600**. In some embodiments, liquid PFPE precursor material **602** is disposed on

patterned substrate **600** via a spin-coating process. Liquid PFPE precursor material **602** is treated by treating process T_{r1} to form a layer of treated liquid PFPE precursor material **604**.

Referring now to Figure 6B, the layer of treated liquid PFPE precursor material **604** is removed from patterned substrate **600**. In some embodiments, the layer of treated liquid PFPE precursor material **604** is contacted with substrate **606**. In some embodiments, substrate 606 comprises a planar substrate or a substantially planar substrate. In some embodiments, the layer of treated liquid PFPE precursor material is treated by treating process T_{r2}, to form two-layer assembly **608**.

Referring now to Figure 6C, a predetermined volume of degradable or selectively soluble material **610** is disposed on two-layer assembly **608**. In some embodiments, the predetermined volume of degradable or selectively soluble material **610** is disposed on two-layer assembly **608** via a spin-coating process. Referring once again to Figure 6C, liquid precursor material **602** is disposed on two-layer assembly **608** and treated to form a layer of PFPE material **612**, which covers the predetermined volume of degradable or selectively soluble material **610**.

Referring now to Figure 6D, the predetermined volume of degradable or selectively soluble material **610** is treated by treating process T_{r3} to remove the predetermined volume of degradable or selectively soluble material **610**, thereby forming microstructure **616**. In some embodiments, microstructure **616** comprises a microfluidic channel. In some embodiments, treating process T_{r3} is selected from the group consisting of a thermal process, an irradiation process, and a dissolution process.

In some embodiments, patterned substrate **600** comprises an etched silicon wafer. In some embodiments, the patterned substrate comprises a photoresist patterned substrate. For the purposes of the presently disclosed subject matter, the patterned substrate can be fabricated by any of the processing methods known in the art, including, but not limited to, photolithography, electron beam lithography, and ion milling.

In some embodiments, degradable or selectively soluble material **610** is selected from the group consisting of a polyolefin sulfone, a cellulose fiber,

a polylactone, and a polyelectrolyte. In some embodiments, the degradable or selectively soluble material **610** is selected from a material that can be degraded or dissolved away. In some embodiments, degradable or selectively soluble material **610** is selected from the group consisting of a salt, a water-soluble polymer, and a solvent-soluble polymer.

In addition to simple channels, the presently disclosed subject matter also provides for the fabrication of multiple complex structures that can be "injection molded" or fabricated ahead of time and embedded into the material and removed as described above.

Figures 7A-C illustrate an embodiment of the presently disclosed method for forming a microchannel or a microstructure through the use of a sacrificial layer. Referring now to Figure 7A, a substrate **700** is provided. In some embodiments, substrate **700** is coated with a liquid PFPE precursor material **702**. Sacrificial structure **704** is placed on substrate **700**. In some embodiments, liquid PFPE precursor material **702** is treated by treating process T_{r1}.

Referring now to Figure 7B, a second liquid PFPE precursor material **706** is disposed over sacrificial structure **704**, in such a way to encase sacrificial structure **704** in second liquid precursor material **706**. Second liquid precursor material **706** is then treated by treating process T_{r2}. Referring now to Figure 7C, sacrificial structure **704** is treated by treating process T_{r3}, to degrade and/or remove sacrificial structure, thereby forming microstructure **708**. In some embodiments, microstructure **708** comprises a microfluidic channel.

In some embodiments, substrate **700** comprises a silicon wafer. In some embodiments, sacrificial structure **704** comprises a degradable or selectively soluble material. In some embodiments, sacrificial structure **704** is selected from the group consisting of a polyolefin sulfone, a cellulose fiber, a polylactone, and a polyelectrolyte. In some embodiments, the sacrificial structure **704** is selected from a material that can be degraded or dissolved away. In some embodiments, sacrificial structure **704** is selected from the group consisting of a salt, a water-soluble polymer, and a solvent-soluble polymer.

X. Microfluidics unit operations

Microfluidic control devices are necessary for the development of effective lab-on-a-chip operations. Valve structures and actuation, fluid control, mixing, separation, and detection at microscale levels must be designed to have a large-scale shift to miniaturization. To construct such devices, integration of the individual components on a common platform must be developed so that solvents and solutes can be completely controlled.

Microfluidic flow controllers are traditionally externally pump-based, including hydrodynamic, reciprocating, acoustic, and peristaltic pumps, and can be as simple as a syringe (see U.S. Patent No. 6,444,106 to Mcbride et al., U.S. Patent No. 6,811,385 to Blakley, U.S. Published Patent Application No. 20040028566 to Ko et al.). More recently, electroosmosis, a process that does not require moving parts, has experienced success as a fluid flow driver (see U.S. Patent No. 6,406,605 to Moles, U.S. Patent No. 6,568,910 to Parse). Other fluid flow devices that do not require moving parts use gravity (see U.S. Patent No. 6,743,399 to Weigl et al.), centrifugal force (see U.S. Patent No. 6,632,388 to Sanae et al.), capillary action (see U.S. Patent No. 6,591,852 to McNeely et al.), or heat (see U.S. Published Patent Application No. 20040257668 to Ito) to drive liquids through the microchannels. Other inventions create liquid flow by the application of an external force, such as a blade (see U.S. Patent No. 6,068,751 to Neukermans).

Valves also are used in fluid flow control. Valves can be actuated by applying an external force, such as a blade, cantilever, or plug to an elastomeric channel (see U.S. Patent No. 6,068,751 to Neukermans). Elastic channels also can contain membranes that can be deflected by air pressure and/or liquid pressure, e.g., water pressure, electrostatically, or magnetically (see U.S. Patent No. 6,408,878 to Unger et al.). Other 2-way valves are actuated by light (see U.S. Published Patent Application No. 20030156991 to Halas et al.), piezoelectric crystals (see Published PCT International Application No. WO 2003/089,138 to Davis et al.), particle deflection (see U.S. Patent No. 6,802,489 to Marr et al.), or bubbles formed within the channel electrochemically (see Published PCT International Application No. WO

2003/046,256 to Hua et al.). One-way or "check valves" also can be formed in microchannels with balls, flaps, or diaphragms (see U.S. Patent No. 6,817,373 to Cox et al.; U.S. Patent No. 6,554,591 to Dai et al.; Published PCT International Application No. WO 2002/053,290 to Jeon et al.). Rotary-type switching valves are used for complex reactions (see Published PCT International Application No. WO 2002/055,188 to Powell et al.).

Microscale mixing and separation components are necessary to facilitate reactions and evaluate products. In microfluidic devices, mixing is most often done by diffusion, in channels of long length scales, curved, with variable widths, or having features that cause turbulence (see U.S. Patent No. 6,729,352 to O'Conner et al., U.S. Published Patent Application No. 20030096310 to Hansen et al.). Mixing also can be accomplished electroosmotically (see U.S. Patent No. 6,482,306 to Yager et al.) or ultrasonically (see U.S. Patent No. 5,639,423 to Northrup et al.). Separations in micro-scale channels typically use three methods: electrophoresis, packed columns or gel within a channel, or functionalization of channel walls. Electrophoresis is commonly done with charged molecules, such as nucleic acids, peptides, proteins, enzymes, and antibodies and the like, and is the simplest technique (see U.S. Patent No. 5,958,202 to Regnier et al., U.S. Patent No. 6,274,089 to Chow et al.). Channel columns can be packed with porous or stationary-phase coated beads or a gel to facilitate separations (see Published PCT International Application No. WO 2003/068,402 to Koehler et al., U.S. Published Patent Application No. 20020164816 to Quake et al., U.S. Patent No. 6,814,859 to Koehler et al.). Possible packing materials include silicates, talc, Fuller's earth, glass wool, charcoal, activated charcoal, celite, silica gel, alumina, paper, cellulose, starch, magnesium silicate, calcium sulfate, silicic acid, florisil, magnesium oxide, polystyrene, p-aminobenzyl cellulose, polytetrafluoroethylene resin, polystyrene resin, SEPHADEX™ (Amersham Biosciences, Corp., Piscataway, New Jersey, United States of America), SEPHAROSE™ (Amersham Biosciences, Corp., Piscataway, New Jersey, United States of America), controlled pore glass beads, agarose, other solid resins known to one skilled in the art and combinations of two or more of any of the foregoing. Magnetizable material, such as ferric oxide,

nickel oxide, barium ferrite or ferrous oxide, also can be imbedded, encapsulated of otherwise incorporated into a solid-phase packing material.

The walls of microfluidic chambers also can be functionalized with a variety of ligands that can interact or bind to an analyte or to a contaminant in an analyte solution. Such ligands include: hydrophilic or hydrophobic small molecules, steroids, hormones, fatty acids, polymers, RNA, DNA, PNA, amino acids, peptides, proteins (including antibody binding proteins such as protein G), antibodies or antibody fragments (FABs, etc), antigens, enzymes, carbohydrates (including glycoproteins or glycolipids), lectins, cell surface receptors (or portions thereof), species containing a positive or a negative charge, and the like (see U.S. Published Patent Application No. 20040053237 to Liu et al., Published PCT International Application No. WO 2004/007,582 to Augustine et al., U.S. Published Patent Application No. 20030190608 to Blackburn).

Thus, in some embodiments, the presently disclosed subject matter describes a method of flowing a material and/or mixing two or more materials in a PFPE-based microfluidic device. In some embodiments, the presently disclosed subject matter describes a method of conducting a chemical reaction, including but not limited to synthesizing a biopolymer, such as DNA. In some embodiments, the presently disclosed subject matter describes a method of screening a sample for a characteristic. In some embodiments, the presently disclosed subject matter describes a method of dispensing a material. In some embodiments, the presently disclosed subject matter describes a method of separating a material.

X.A. Method of Flowing a Material and/or Mixing Two Materials in a PFPE-based Microfluidic Device

Referring now to Figure 8, a schematic plan view of a microfluidic device of the presently disclosed subject matter is shown. The microfluidic device is referred to generally at **800**. Microfluidic device **800** comprises a patterned layer **802**, and a plurality of holes **810A**, **810B**, **810C**, and **810D**. These holes can be further described as inlet aperture **810A**, inlet aperture **810B**, and inlet aperture **810C**, and outlet aperture **810D**. Each of apertures

810A, **810B**, **810C**, and **810D** are covered by seals **820A**, **820B**, **820C**, and **820D**, which are preferably reversible seals. Seals **820A**, **820B**, **820C**, and **820D** are provided so that materials, including but not limited to, solvents, chemical reagents, components of a biochemical system, samples, inks, and reaction products and/or mixtures of solvents, chemical reagents, components of a biochemical system, samples, inks, reaction products and combinations thereof, can be stored, shipped, or otherwise maintained in microfluidic device **800** if desired. Seals **820A**, **820B**, **820C**, and **820D** can be reversible, that is, removable, so that microfluidic device **800** can be implemented in a chemical reaction or other use and then can be resealed if desired.

Continuing with reference to Figure 8, in some embodiments, apertures **810A**, **810B**, and **810C**, further comprise pressure-actuated valves (comprising intersecting, overlaid flow channels not shown) which can be actuated to seal the microfluidic channel associated with the aperture.

Continuing with reference to Figure 8, patterned layer **802** of microfluidic device **800** comprises an integrated network **830** of microscale channels. Optionally, pattern layer **802** comprises a functionalized surface, such as that shown in Figure 5A. Integrated network **830** can comprise a series of fluidly connected microscale channels designated by the following reference characters: **831**, **832**, **833**, **834**, **835**, **836**, **837**, **838**, **839**, and **840**. Thus, inlet aperture **810A** is in fluid communication with microscale channel **831** that extends away from aperture **810A** and is in fluid communication with microscale channel **832** via a bend. In integrated network **830** depicted in Figure 8, a series of 90° bends are shown for convenience. It is noted, however, that the paths and bends provided in the channels of integrated network **830**, can encompass any desired configuration, angle, or other characteristic (such as but not limited to a serpentine section). Indeed, fluid reservoirs **850A** and **850B** can be provided along microscale channels **831**, **832**, **833**, and **834**, respectively, if desired. As shown in Figure 8, fluid reservoirs **850A** and **850B** comprise at least one dimension that is greater than a dimension of the channels that are immediately adjacent to them.

Continuing, then, with reference to Figure 8, microscale channels **832** and **834** intersect at intersecting point **860A** and proceed into a single

microscale channel **835**. Microscale channel **835** proceeds to a chamber **870**, which in the embodiment shown in Figure 8, is dimensioned to be wider than microscale channel **835**. In some embodiments, chamber **870** comprises a reaction chamber. In some embodiments, chamber **870** comprises a mixing region. In some embodiments, chamber **870** comprises a separation region. In some embodiments, the separation region comprises a given dimension, e.g., length, of a channel, wherein the material is separated by charge, or mass, or combinations thereof, or any other physical characteristic wherein a separation can occur over a given dimension. In some embodiments, the separation region comprises an active material **880**. As would be understood by one of ordinary skill in the art, the term "active material" is used herein for convenience and does not imply that the material must be activated to be used for its intended purpose. In some embodiments, the active material comprises a chromatographic material. In some embodiments, the active material comprises a target material.

Continuing with Figure 8, it is noted that chamber **870** does not necessarily need to be of a wider dimension than an adjacent microscale channel. Indeed chamber **870** can simply comprise a given segment of a microscale channel wherein at least two materials are separated, mixed, and/or reacted. Extending from chamber **870** substantially opposite from microscale channel **835** is microscale channel **836**. Microscale channel **836** forms a T-junction with microscale channel **837**, which extends away from and is in fluid communication with aperture **810C**. Thus, the junction of microscale channels **836** and **837** form intersecting point **860B**. Microscale channel **838** extends from intersecting point **860B** in a direction substantially opposite microscale channel **837** and to fluid reservoir **850C**. Fluid reservoir **850C** is dimensioned to be wider than microscale channel **838** for a predetermined length. As noted above, however, a given section of a microscale channel can act as a fluid reservoir without the need to necessarily change a dimension of the section of microscale channel. Moreover, microscale channel **838** could act as a reaction chamber in that a reagent flowing from microscale channel **837** to intersection point **860B** could react with a reagent moving from microscale channel **836** to intersection point **860B** and into

microscale channel **838**.

Continuing with reference to Figure 8, microscale channel **839** extends from fluid reservoir **850C** substantially opposite microfluidic channel **838** and travels through a bend into microscale channel **840**. Microscale channel **840** is fluidly connected to outlet aperture **810D**. Outlet aperture **810D** can optionally be reversibly sealed via seal **820D**, as discussed above. Again, the reversible sealing of outlet aperture **810D** can be desirable in the case of an embodiment where a reaction product is formed in microfluidic device **800** and is desired to be transported to another location in microfluidic device **800**.

The flow of a material can be directed through the integrated network **830** of microscale channels, including channels, fluid reservoirs, and reaction chambers through the use of pressure-actuated valves and the like known in the art, for example those described in U.S. Patent No. 6,408,878 to Unger et al., which is incorporated herein by reference in its entirety. The presently disclosed subject matter thus provides a method of flowing a material through a PFPE-based microfluidic device. In some embodiments, the method comprises providing a microfluidic device comprising (i) a perfluoropolyether (PFPE) material having a characteristic selected from the group consisting of: a viscosity greater than about 100 centistokes (cSt); a viscosity less than about 100 cSt, provided that the liquid PFPE precursor material having a viscosity less than 100 cSt is not a free-radically photocurable PFPE material; (ii) a functionalized PFPE material; (iii) a fluoroolefin-based elastomer; and (iv) combinations thereof, and wherein the microfluidic device comprises one or more microscale channels; and flowing a material in the microscale channel.

Also provided is a method of mixing two or more materials. In some embodiments, the method comprises providing a microscale device comprising (i) a perfluoropolyether (PFPE) material having a characteristic selected from the group consisting of: a viscosity greater than about 100 centistokes (cSt); a viscosity less than about 100 cSt, provided that the liquid PFPE precursor material having a viscosity less than 100 cSt is not a free-radically photocurable PFPE material; (ii) a functionalized PFPE material; (iii) a fluoroolefin-based elastomer; and (iv) combinations thereof; and contacting a first material and a second material in the device to mix the first and second

materials. Optionally, the microscale device is selected from the group consisting of a microfluidics device and a microtiter plate.

In some embodiments, the method comprises disposing a material in the microfluidic device. In some embodiments, as is best shown in Figure 10 and as discussed in more detail herein below, the method comprises applying a driving force to move the material along the microscale channel.

In some embodiments, the layer of PFPE material covers a surface of at least one of the one or more microscale channels. Optionally, the layer of PFPE material comprises a functionalized surface. In some embodiments, the microfluidic device comprises one or more patterned layers of PFPE material, and wherein the one or more patterned layers of the PFPE material defines the one or more microscale channels. In this case the patterned layer of PFPE can comprise a functionalized surface. In some embodiments, the microfluidic device can further comprise a patterned layer of a second polymeric material, wherein the patterned layer of the second polymeric material is in operative communication with the at least one of the one or more patterned layers of PFPE material. See Figure 2.

In some embodiments, the method comprises at least one valve. In some embodiments the valve is a pressure-actuated valve, wherein the pressure-actuated valve is defined by one of: (a) a microscale channel; and (b) at least one of the plurality of holes. In some embodiments, the pressure-actuated valve is actuated by introducing a pressurized fluid into one of: (a) a microscale channel; and (b) at least one of the plurality of holes.

In some embodiments, the pressurized fluid has a pressure between about 10 psi and about 40 psi. In some embodiments, the pressure is about 25 psi. In some embodiments, the material comprises a fluid. In some embodiments, the fluid comprises a solvent. In some embodiments, the solvent comprises an organic solvent. In some embodiments, the material flows in a predetermined direction along the microscale channel.

In the case of mixing two materials, which in some embodiments can comprise mixing two reactants to provide a chemical reaction, the contacting of the first material and the second material is performed in a mixing region defined in the one or more microscale channels. The mixing region can

comprise a geometry selected from the group consisting of a T-junction, a serpentine, an elongated channel, a microscale chamber, and a constriction. Optionally, the first material and the second material are disposed in separate channels of the microfluidic device. Also, the contacting of the first material and the second material can be performed in a mixing region defined by an intersection of the channels.

Continuing with a method of mixing, the method can comprise flowing the first material and the second material in a predetermined direction in the microfluidic device, and can comprise flowing the mixed materials in a predetermined direction in the microfluidic device. In some embodiments, the mixed material can be contacted with a third material to form a second mixed material. In some embodiments the mixed material comprises a reaction product and the reaction product can be subsequently reacted with a third reagent. One of ordinary skill in the art upon review of the presently disclosed subject matter would recognize that the description of the method of mixing provided immediately hereinabove is for the purposes of illustration and not limitation. Accordingly, the presently disclosed method of mixing materials can be used to mix a plurality of materials and form a plurality of mixed materials and/or a plurality of reaction products. The mixed materials, including but not limited to reaction products, can be flowed to an outlet aperture of the microfluidic device. A driving force can be applied to move the materials through the microfluidic device. See Figure 10. In some embodiments the mixed materials are recovered.

In an embodiment employing a microtiter plate, the microtiter plate can comprise one or more wells. In some embodiments, the layer of PFPE material covers a surface of at least one of the one or more wells. The layer of PFPE material can comprise a functionalized surface. See Figure 5B.

X.B. Method of Synthesizing a Biopolymer in a PFPE-based Microfluidic Device

In some embodiments, the presently disclosed PFPE-based microfluidic device can be used in biopolymer synthesis, for example, in synthesizing oligonucleotides, proteins, peptides, DNA, and the like. In some

embodiments, such biopolymer synthesis systems comprise an integrated system comprising an array of reservoirs, fluidic logic for selecting flow from a particular reservoir, an array of channels, reservoirs, and reaction chambers in which synthesis is performed, and fluidic logic for determining into which channels the selected reagent flows.

Referring now to Figure 9, a plurality of reservoirs, e.g., reservoirs **910A**, **910B**, **910C**, and **910D**, have bases **A**, **C**, **T**, and **G** respectively disposed therein, as shown. Four flow channels **920A**, **920B**, **920C**, and **920D** are connected to reservoirs **910A**, **910B**, **910C**, and **910D**. Four control channels **922A**, **922B**, **922C**, and **922D** (shown in phantom) are disposed thereacross with control channel **922A** permitting flow only through flow channel **920A** (i.e., sealing flow channels **920B**, **920C**, and **920D**), when control channel **922A** is pressurized. Similarly, control channel **922B** permits flow only through flow channel **920B** when pressurized. As such, the selective pressurization of control channels **922A**, **922B**, **922C**, and **922D** sequentially selects a desired base **A**, **C**, **T**, and **G** from a desired reservoir **910A**, **910B**, **910C**, or **910D**. The fluid then passes through flow channel **920E** into a multiplexed channel flow controller **930**, (including, for example, any system as shown in Figure 8) which in turn directs fluid flow into one or more of a plurality of synthesis channels or reaction chambers **940A**, **940B**, **940C**, **940D**, or **940E** in which solid phase synthesis can be carried out.

In some embodiments, instead of starting from the desired base **A**, **C**, **T**, and **G**, a reagent selected from one of a nucleotide and a polynucleotide is disposed in at least one of reservoir **910A**, **910B**, **910C**, and **910D**. In some embodiments, the reaction product comprises a polynucleotide. In some embodiments, the polynucleotide is DNA.

Accordingly, after a review of the present disclosure, one of ordinary skill in the art would recognize that the presently disclosed PFPE-based microfluidic device can be used to synthesize biopolymers, as described in U.S. Patent Nos. 6,408,878 to Unger et al. and 6,729,352 to O'Conner et al., and/or in a combinatorial synthesis system as described in U.S. Patent No. 6,508,988 to van Dam et al., each of which is incorporated herein by reference in its entirety.

X.C. Method of Incorporating a PFPE-based Microfluidic Device into an Integrated Fluid Flow System.

In some embodiments, the method of performing a chemical reaction or flowing a material within a PFPE-based microfluidic device comprises incorporating the microfluidic device into an integrated fluid flow system. Referring now to Figure 10, a system for carrying out a method of flowing a material in a microfluidic device and/or a method of performing a chemical reaction in accordance with the presently disclosed subject matter is schematically depicted. The system itself is generally referred to at **1000**. System **1000** can comprise a central processing unit **1002**, one or more driving force actuators **1010A**, **1010B**, **1010C**, and **1010D**, a collector **1020,** and a detector **1030**. In some embodiments, detector **1030** is in fluid communication with the microfluidic device (shown in shadow). System microfluidic device **1000** of Figure 8, and these reference numerals of Figure 8 are employed in Figure 10. Central processing unit (CPU) **1002** can be, for example, a general purpose personal computer with a related monitor, keyboard or other desired user interface. Driving force actuators **1010A**, **1010B**, **1010C**, and **1010D** can be any suitable driving force actuator as would be apparent to one of ordinary skill in the art upon review of the presently disclosed subject matter. For example, driving force actuators **1010A**, **1010B**, **1010C**, and **1010D** can be pumps, electrodes, injectors, syringes, or other such devices that can be used to force a material through a microfluidic device. Representative driving forces themselves thus include capillary action, pump driven fluid flow, electrophoresis based fluid flow, pH gradient driven fluid flow, or other gradient driven fluid flow.

In the schematic of Figure 10 driving force actuator **1010D** is shown as connected at outlet aperture **810D**, as will be described below, to demonstrate that at least a portion of the driving force can be provided at the end point of the desired flow of solution, reagent, and the like. Collector **1020** also is provided to show that a reaction product **1048**, as discussed below, can be collected at the end point of system flow. In some embodiments, collector **1020** comprises a fluid reservoir. In some embodiments, collector **1020**

comprises a substrate. In some embodiments, collector **1020** comprises a detector. In some embodiments, collector **1020** comprises a subject in need of therapeutic treatment. For convenience, system flow is generally represented in Figure 10 by directional arrows **F1**, **F2**, and **F3**.

Continuing with reference to Figure 10, in some embodiments a chemical reaction is performed in integrated flow system **1000**. In some embodiments, material **1040**, e.g, a chemical reagent, is introduced to microfluidic device **1000** through aperture **810A**, while a second material **1042**, e.g., a second chemical reagent, is introduced to microfluidic device **1000**, via inlet aperture **810B**. Optionally, microfluidics device **1000** comprises a functionalized surface (see Figure 5A). Driving force actuators **1010A** and **1010B** propel chemical reagents **1040** and **1042** to microfluidic channels **831** and **833**, respectively. Flow of chemical reagents **1040** and **1042** continues to fluid reservoirs **850A** and **850B**, where a reserve of reagents **1040** and **1042** is collected. Flow of chemical reagents **1040** and **1042** continues into microfluidic channels **832** and **834** to intersection point **860A** wherein initial contact between chemical reagents **1040** and **1042** occurs. Flow of chemical reagents **1040** and **1042** then continues to reaction chamber **870** where a chemical reaction between chemical reagents **1040** and **1042** proceeds.

Continuing with reference to Figure 10, reaction product **1044** flows to microscale channel **836** and to intersection point **860B**. Chemical reagent **1046** then reacts with reaction product **1044** beginning at intersection point **860B** through reaction chamber **838** and to fluid reservoir **850C.** A second reaction product **1048** is formed. Flow of the second reaction product **1048** continues through microscale channel **840** to aperture **810D** and finally into collector **1020**. Thus, it is noted that CPU **1002** actuates driving force actuator **1010C** such that chemical reagent **1046** is released at an appropriate time to contact reaction product **1044** at intersection point **860B**.

X.D. Representative Applications of a Microfluidic Device

In some embodiments, the presently disclosed subject matter discloses a method of screening a sample for a characteristic. In some embodiments, the presently disclosed subject matter discloses a method of dispensing a material. In some embodiments, the presently disclosed subject matter discloses a method of separating a material. Accordingly, one of ordinary skill in the art would recognize that a microfluidic device described herein can be applied to many applications, including, but not limited to, genome mapping, rapid separations, sensors, nanoscale reactions, ink-jet printing, drug delivery, Lab-on-a-Chip, in vitro diagnostics, injection nozzles, biological studies, high-throughput screening technologies, such as for use in drug discovery and materials science, diagnostic and therapeutic tools, research tools, and the biochemical monitoring of food and natural resources, such as soil, water, and/or air samples collected with portable or stationary monitoring equipment.

X.D.1. Method of Screening a Sample for a Characteristic

In some embodiments, the presently disclosed subject matter discloses a method of screening a sample for a characteristic. In some embodiments, the method comprises:

(a) providing a microscale device comprising:

(i) a perfluoropolyether (PFPE) material having a characteristic selected from the group consisting of: a viscosity greater than about 100 centistokes (cSt) and a viscosity less than about 100 cSt, provided that the liquid PFPE precursor material having a viscosity less than 100 cSt is not a free-radically photocurable PFPE material;

(ii) a functionalized PFPE material;

(iii) a fluoroolefin-based elastomer; and

(iv) combinations thereof;

(b) providing a target material;

(c) disposing the sample in the microscale device;

(d) contacting the sample with the target material; and

(e) detecting an interaction between the sample and the target,

wherein the presence or the absence of the interaction is indicative of the characteristic of the sample.

Referring once again to Figure 10, at least one of materials **1040** and **1042** comprises a sample. In some embodiments, at least one of materials **1040** and **1042** comprises a target material. Thus, a "sample" generally refers to any material about which information relating to a characteristic is desired. Also, a "target material" can refer to any material that can be used to provide information relating to a characteristic of a sample based on an interaction between the target material and the sample. In some embodiments, for example, when sample **1040** contacts target material **1042** an interaction occurs. In some embodiments, the interaction produces a reaction product **1044**. In some embodiments, the interaction comprises a binding event. In some embodiments, the binding event comprises the interaction between, for example, an antibody and an antigen, an enzyme and a substrate, or more particularly, a receptor and a ligand, or a catalyst and one or more chemical reagents. In some embodiments, the reaction product is detected by detector **1030**.

In some embodiments, the method comprises disposing the target material in at least one of the plurality of channels. Referring once again to Figure 10, in some embodiments, the target material comprises active material **880**. In some embodiments, the target material, the sample, or both the target and the sample are bound to a functionalized surface. In some embodiments, the target material comprises a substrate, for example a non-patterned layer. In some embodiments, the substrate comprises a semiconductor material. In some embodiments, at least one of the plurality of channels of the microfluidic device is in fluid communication with the substrate, e.g., a non-patterned layer. In some embodiments, the target material is disposed on a substrate, e.g., a non-patterned layer. In some embodiments, at least one of the one or more channels of the microfluidic device is in fluid communication with the target material disposed on the substrate.

In some embodiments, the method comprises disposing a plurality of samples in at least one of the plurality of channels. In some embodiments, the sample is selected from the group consisting of a therapeutic agent, a diagnostic agent, a research reagent, a catalyst, a metal ligand, a non-biological organic material, an inorganic material, a foodstuff, soil, water, and air. In some embodiments, the sample comprises one or more members of one or more libraries of chemical or biological compounds or components. In some embodiments, the sample comprises one or more of a nucleic acid template, a sequencing reagent, a primer, a primer extension product, a restriction enzyme, a PCR reagent, a PCR reaction product, or a combination thereof. In some embodiments, the sample comprises one or more of an antibody, a cell receptor, an antigen, a receptor ligand, an enzyme, a substrate, an immunochemical, an immunoglobulin, a virus, a virus binding component, a protein, a cellular factor, a growth factor, an inhibitor, or a combination thereof.

In some embodiments, the target material comprises one or more of an antigen, an antibody, an enzyme, a restriction enzyme, a dye, a fluorescent dye, a sequencing reagent, a PCR reagent, a primer, a receptor, a ligand, a chemical reagent, or a combination thereof.

In some embodiments, the interaction comprises a binding event. In some embodiments, the detecting of the interaction is performed by at least one or more of a spectrophotometer, a fluorometer, a photodiode, a photomultiplier tube, a microscope, a scintillation counter, a camera, a CCD camera, film, an optical detection system, a temperature sensor, a conductivity meter, a potentiometer, an amperometric meter, a pH meter, or a combination thereof.

Accordingly, after a review of the present disclosure, one of ordinary skill in the art would recognize that the presently disclosed PFPE-based microfluidic device can be used in various screening techniques, such as those described in U.S. Patent Nos. 6,749,814 to Bergh et al., 6,737,026 to Bergh et al., 6,630,353 to Parce et al., 6,620,625 to Wolk et al., 6,558,944 to Parce et al., 6,547,941 to Kopf-Sill et al., 6,529,835 to Wada et al., 6,495,369 to Kercso et al., and 6,150,180 to Parce et al., each of which is incorporated

by reference in its entirety. Further, after a review of the present disclosure, one of ordinary skill in the art would recognize that the presently disclosed PFPE-based microfluidic device can be used, for example, to detect DNA, proteins, or other molecules associated with a particular biochemical system, as described in U.S. Patent No. 6,767,706 to Quake et al., which is incorporated herein by reference in its entirety.

X.D.2. Method of Dispensing a Material

Additionally, the presently disclosed subject matter describes a method of dispensing a material. In some embodiments, the method comprises:

(a) providing a microfluidic device comprising:

(i) a perfluoropolyether (PFPE) material having a characteristic selected from the group consisting of: a viscosity greater than about 100 centistokes (cSt) and a viscosity less than about 100 cSt, provided that the liquid PFPE precursor material having a viscosity less than 100 cSt is not a free-radically photocurable PFPE material;

(ii) a functionalized PFPE material;

(iii) a fluoroolefin-based elastomer; and

(iv) combinations thereof; and wherein the microfluidics device comprises one or more microscale channels, and wherein at least one of the one or more microscale channels comprises an outlet aperture;

(b) providing at least one material;

(c) disposing at least one material in at least one of the one or more microscale channels; and

(d) dispensing at least one material through the outlet aperture.

In some embodiments, the layer of PFPE material covers a surface of at least one of the one or more microscale channels.

Referring once again to Figure 10, in some embodiments, a material, e.g., material **1040**, second material **1042**, chemical reagent **1046**, reaction product **1044**, and/or reaction product **1048** flow through outlet aperture **810D** and are dispensed in or on collector **1020**. In some embodiments, the target material, the sample, or both the target and the sample are bound to a functionalized surface.

In some embodiments, the material comprises a drug. In some embodiments, the method comprises metering a predetermined dosage of the drug. In some embodiments, the method comprises dispensing the predetermined dosage of the drug.

In some embodiments, the material comprises an ink composition. In some embodiments, the method comprises dispensing the ink composition on a substrate. In some embodiments, the dispensing of the ink composition on a substrate forms a printed image.

Accordingly, after a review of the present disclosure, one of ordinary skill in the art would recognize that the presently disclosed PFPE-based microfluidic device can be used for microfluidic printing as described in U.S. Patent Nos. 6,334,676 to Kaszczuk et al., 6,128,022 to DeBoer et al., and 6,091,433 to Wen, each of which is incorporated herein by reference in its entirety.

X.D.3 Method of Separating a Material

In some embodiments, the presently disclosed subject matter describes a method of separating a material, the method comprising:

(a) providing a microfluidic device comprising:

(i) a perfluoropolyether (PFPE) material having a characteristic selected from the group consisting of: a viscosity greater than about 100 centistokes (cSt) and a viscosity less than about 100 cSt, provided that the liquid PFPE precursor material having a viscosity less than 100 cSt is not a free-radically photocurable PFPE material;

(ii) a functionalized PFPE material;

(iii) a fluoroolefin-based elastomer; and

(iv) combinations thereof; and wherein the microfluidics device comprises one or more microscale channels, and wherein at least one of the one or more microscale channels comprises a separation region;

(b) disposing a mixture comprising at least a first material and a second material in the microfluidic device;

(c) flowing the mixture through the separation region; and

(d) separating the first material from the second material in the separation region to form at least one separated material.

Referring once again to Figure 10, in some embodiments, at least one of material **1040** and second material **1042** comprise a mixture. For example, material **1040**, e.g., a mixture, flows through the microfluidic system to chamber **870**, which in some embodiments comprises a separation region. In some embodiments, the separation region comprises active material **880**, e.g., a chromatographic material. Material **1040**, e.g., a mixture, is separated in chamber **870**, e.g., a separation chamber, to form a third material **1044**, e.g., a separated material. In some embodiments, separated material **1044** is detected by detector **1030**.

In some embodiments, the separation region comprises a chromatographic material. In some embodiments, the chromatographic material is selected from the group consisting of a size-separation matrix, an affinity-separation matrix, and a gel-exclusion matrix, or a combination thereof.

In some embodiments, the first or second material comprises one or more members of one or more libraries of chemical or biological compounds or components. In some embodiments, the first or second material comprises one or more of a nucleic acid template, a sequencing reagent, a primer, a primer extension product, a restriction enzyme, a PCR reagent, a PCR reaction product, or a combination thereof. In some embodiments, the first or

second material comprises one or more of an antibody, a cell receptor, an antigen, a receptor ligand, an enzyme, a substrate, an immunochemical, an immunoglobulin, a virus, a virus binding component, a protein, a cellular factor, a growth factor, an inhibitor, or a combination thereof.

In some embodiments, the method comprises detecting the separated material. In some embodiments, the detecting of the separated material is performed by at least one or more of a spectrophotometer, a fluorometer, a photodiode, a photomultiplier tube, a microscope, a scintillation counter, a camera, a CCD camera, film, an optical detection system, a temperature sensor, a conductivity meter, a potentiometer, an amperometric meter, a pH meter, or a combination thereof.

Accordingly, after a review of the present disclosure, one of ordinary skill in the art would recognize that the presently disclosed PFPE-based microfluidic device can be used to separate materials, as described in U.S. Patent Nos. 6,752,922 to Huang et al., 6,274,089 to Chow et al., and 6,444,461 to Knapp et al., each of which is incorporated herein by reference in its entirety.

XI. Applications for Functionalized Microfluidic Devices

Fluidic microchip technologies are increasingly being used as replacements for traditional chemical and biological laboratory functions. Microchips that perform complex chemical reactions, separations, and detection on a single device have been fabricated. These "lab-on-a-chip" applications facilitate fluid and analyte transport with the advantages of reduced time and chemical consumption and ease of automation.

A variety of biochemical analysis, reactions, and separations have been performed within microchannel systems. High throughput screening assays of synthesized molecules and natural products are of great interest. Microfluidic devices for screening a wide variety of molecules based on their ability to inhibit the interactions of enzymes and fluorescently labeled substrates have been described (U.S. Patent No. 6,046,056, to Parse et al.). As described by Parse et al., such devices allow for screening natural or synthetic libraries of potential drugs through their antagonist or agonist

properties. The types of molecules that can be screened include, but are not limited to, small organic or inorganic molecules, polysaccharides, peptides, proteins, nucleic acids or extracts of biological materials such as bacteria, fungi, yeast, plants and animal cells. The analyte compounds can be free in solution or attached to a solid support, such as agarose, cellulose, dextran, polystyrene, carboxymethyl cellulose, polyethylene glycol (PEG), filter paper, nitrocellulose, ion exchange resins, plastic films, glass beads, polyaminemethylvinylether maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, and the like. Compounds can be tested as pure compounds or in pools. For example, U.S. Patent No. 6,007,690 to Nelson et al. relates to a microfluidic molecular diagnostic that purifies DNA from whole blood samples. The device uses an enrichment channel that cleans up or concentrates the analyte sample. For example, the enrichment channel could hold antibody coated beads to remove various cell parts via their antigenic components or could hold chromatographic components, such as ion exchange resin or a hydrophobic or hydrophilic membrane. The device also can comprise a reactor chamber, wherein various reactions can be performed on the analyte, such as a labeling reaction or in the case of a protein analyte, a digestion reaction. Further, U.S. Published Patent Application No. 20040256570 to Beebe et al. describes a device where antibody interaction with an antigenic analyte material coated on the outside of a liposome is detected when that interaction causes the lysis of the liposome and its release of a detectable molecule. U.S. Published Patent Application No. 20040132166 to Miller et al. provides a microfluidic device that can sense environmental factors, such as pH, humidity, and O_2 levels critical for the growth of cells. The reaction chambers in these devices can function as bioreactors capable of growing cells, allowing for their use to transfect cells with DNA and produce proteins, or to test for the possible bioavailability of drug substances by measuring their absorbance across CACO-2 cell layers.

In addition of growing cells, microfluidic devices also have been used to sort cells. U.S. Patent No. 6,592,821 to Wada et al. describes hydrodynamic focusing to sort cells and subcellular components, including

individual molecules, such as nucleic acids, polypeptides or other organic molecules, or larger cell components like organelles. The method can sort for cell viability or other cellular expression functions.

Amplification, separation, sequencing, and identification of nucleic acids and proteins are common microfluidic device applications. For example, U.S. Patent No. 5,939,291 to Loewy et al. illustrate a microfluidic device that uses electrostatic techniques to perform isothermal nucleic acid amplification. The device can be used in conjunction with a number of common amplification reaction strategies, including PCR (polymerase chain reaction), LCR (ligase chain reaction), SDA (strand displacement amplification), NASBA (nucleic acid sequence-based amplification), and TMA (transcription-mediated amplification). U.S. Patent No. 5,993,611 to Moroney et al. describes a device that uses capacitive charging to analyze, amplify or otherwise manipulate nucleic acids. Devices have been designed that sort DNA by size, analyzing restriction fragment length polymorphism (see U.S. Patent No. 6,833,242 to Quake et al.). The devices also can have particular use in forensic applications, such as DNA fingerprinting. U.S. Patent No. 6,447,724 to Jensen et al. describes microfluidics that identify components of a mixture based on the different fluorescent lifetimes of the labels attached to members of the mixture. Such a device could be used to analyze sequencing reactions of nucleic acids, proteins or oligosaccharides or to inspect or interrogate members of a combinatorial library of organic molecules.

Other microfluidic devices directed toward specific protein applications include a device that promotes protein crystal growth in microfluidic channels (see U.S. Patent No. 6,409832, to Weigl et al.). In the device, protein sample and solvent are directed to a channel with laminar flow characteristics that form diffusion zones, which provide well-defined crystallization. U.S. Published Patent Application No. 2004/0121449 to Pugia et al. illustrates a device that can separate red blood cells from plasma using minimal centrifugal force on sample sizes as small as 5 microliters. The device could be particularly useful in clinical diagnostics and also could be used to separate any particulate matter from a liquid.

As partly described hereinabove, microfluidic devices have been

utilized as microreactors for a variety of chemical and biological applications. Chambers in these devices can be used for sequencing, restriction enzyme digests, restriction fragment length polymorphism (RFLP) analysis, nucleic acid amplification, or gel electrophoresis (see U.S. Patent No. 6,130,098, to Handique et al.). A multitude of chemical titration reactions can be run in the devices (see U.S. Published Patent Application No. 20040258571, to Lee et al.), including acid-based titrations or titrations based on precipitation (for example, Ag(I) with Cl^-, Br^-, I^-, or SCN^-), complex formation (for example, Ag(I) with CN^-), or redox reactions (such as Fe(II)/Fe(III) with Ce(III)/Ce(IV)). Further, a sensor for potentiometry, amperometry, spectrophotometry, turbidometry, fluorimetry or calorimetry can be attached to the device. Fractionation of proteins (see U.S. Published Patent Application No. 20040245102, to Gilbert et al.) based physical or biological properties is of use in protein expression analysis (finding molecular markers, determining a molecular basis or profile for a disease state or interpreting protein structure/function relationships). A variety of electrophoresis techniques (including capillary isoelectric focusing, capillary zone electrophoresis, and capillary gel electrophoresis) have been employed in microfluidic devices for fractionating proteins (see U.S. Patent No. 6,818,112, to Schneider et al.). The different electrophoretic techniques can be used in series, with or without a labeling step to help with quantitation, and in conjunction with a variety of elution techniques (such as hydrodynamic salt mobilization, pH mobilization, or electroosmotic flow) to further separate proteins. A variety of other materials have been used to aid in separation processes in microfluidic devices. Such materials may be attached to channel walls in a device or be present as a separate matrix inside a channel (see U.S. Patent No. 6,581,441 to Paul; U.S. Patent No. 6,613,581, to Wada et al.). Parallel separation channels can exist to separate many samples at the same time. The solid separation media can be present as a discrete particle or as a porous monolithic solid. Possible materials include silica gel, agarose-based gels, polyacrylamide gels, a colloidal solution, such as a gelatin, starches, non-ionic macroreticular and macroporous resins (such as AMBERCHROM™ (Rohm and Haas Co, Philadelphia, Pennsylvania, United States of America),

AMBERLITE™ (Rohm and Haas Co, Philadelphia, Pennsylvania, United States of America), DOWEX™ (The Dow Chemical Company, Midland, Michigan, United States of America), DUOLITE® (Rohm and Haas Co, Philadelphia, Pennsylvania, United States of America), and the like), or material present as beads (glass, metal, silica, acrylic, SEPHAROSE™, cellulose, ceramic, polymer, and the like). These materials also can have present on their surfaces various biologically based molecules to aid in separation (for example, lectins bind to carbohydrates and antibodies can bind to antigenic groups on different proteins). Membranes within microchannels have been used for electroosmotic separation (see U.S. Patent No. 6,406,605, to Moles). Suitable membranes can be comprised of materials, such as track etched polycarbonate or polyimide.

Temperature, concentration and flow gradients also have been employed to aid in separation in microfluidic devices. U.S. Published Patent Application No. 20040142411 to Kirk et al. discloses the use of chemotaxis (the movement of cells induced by a concentration gradient of a soluble chemotactic stimulus), hapatotaxis (the movement of cells in response to a concentration gradient of a substrate-bound stimulus) and chemoinvasion (the movement of cells into and/or through a barrier or gel matrix in response to a stimulus). Chemotatic stimuli include chemorepellants and chemoattractants. A chemoattractant is any substance that attracts cells. Examples include, but are not limited to, hormones such as epinephrine and vasopressin; immunological agents such as interleukein-2; growth factors, chemokines, cytokines, and various peptides, small molecules and cells. Chemorepellants include irritants such as benzalkonium chloride, propylene glycol, methanol, acetone, sodium dodecyl sulfate, hydrogen peroxide, 1-butanol, ethanol and dimethylsulfoxide; toxins, such as cyanide, carbonylcyanide chlorophenylhydrozone; endotoxins and bacterial lipopolysaccharides; viruses; pathogens; and pyrogens. Non-limiting examples of cells that can be manipulated by these techniques include lymphocytes, monocytes, leukocytes, macrophages, mast cells, T-cells, B-cells, neutrophils, basophils, fibroblasts, tumor cells and many others.

Microfluidic devices as sensors have garnered attention in the last few

years. Such microfluidic sensors can include dye-based detection systems, affinity-based detections systems, microfabricated gravimetric analyzers, CCD cameras, optical detectors, optical microscopy systems, electrical systems, thermocouples, thermoresistors, and pressure sensors. Such devices have been used to detect biomolecules (see Published PCT International Application No. WO 2004/094,986 to Althaus et al.), including polynucleotides, proteins and viruses through their interaction with probe molecules capable of providing an electrochemical signal. For example, intercalation of a nucleic acid sample with a probe molecule, such as doxorubicin can reduce the amount of free doxorubicin in contact with an electrode; and a change in electrical signal results. Devices have been described that contain sensors for detecting and controlling environmental factors inside device reaction chambers such as humidity, pH, dissolved O_2 and dissolved CO_2 (see Published PCT International Application No. WO 2004/069,983 to Rodgers et al.). Such devices have particular use in growing and maintaining cells. The carbon content of samples can be measured in a device (see U.S. Patent No. 6,444,474 to Thomas et al.) wherein UV irradiation oxidizes organics to CO_2, which is then quantitated by conductivity measurements or infrared methods. Capacitance sensors used in microfluidic devices (see Published PCT International Application No. WO 2004/085,063 to Xie et al.) can be used to measure pressure, flow, fluid levels, and ion concentrations.

Another application for microfluidic systems includes the high throughput injection of cells (see Published PCT International Application No. WO 00/20554 to Garman et al.) In such a device, cells are impelled to a needle where they can be injected with a wide variety of materials including molecules and macromolecules, genes, chromosomes, or organelles. The device also can be used to extract material from cells and would be of use in a variety of fields, such as gene therapy, pharmaceutical or agrochemical research, and diagnostics. Microfluidic devices also have been used as a means of delivering ink in ink-jet printing (see U.S. Patent No. 6,575,562 to Anderson et al.), and to direct sample solutions onto an electrospray ionization tip for mass spectrometry (see U.S. Patent No. 6,803,568 to Bousse et al.). Systems for transdermal drug delivery also have been

reported (see Published PCT International Application No. WO 2002/094,368 to Cormier et al.), as well as devices containing light altering elements for use in spectroscopy applications (see U.S. Patent No. 6,498,353 to Nagle et al.).

XII. Applications for Functionalized Microtiter Plates

The presently disclosed materials and methods also can be applied to the design and manufacture of devices to be used in the manner of microtiter plates. Microtiter plates have a variety of uses in the fields of high throughput screening for proteomics, genomics and drug discovery, environmental chemistry assays, parallel synthesis, cell culture, molecular biology and immunoassays. Common base materials used for microtiter plates include hydrophobic materials, such as polystyrene and polypropylene, and hydrophilic materials, such as glass. Silicon, metal, polyester, polyolefin and polytetrafluoroethylene surfaces also have been used for microtiter plates.

Surfaces can be selected for a particular application based on their solvent and temperature compatibilities and for their ability (or lack of ability) to interact with the molecules or biomolecules being assayed or otherwise manipulated. Chemical modification of the base material is often useful in tailoring the microtiter plate to its desired function either by modifying the surface characteristics or by providing a site for the covalent attachment of a molecule or biomolecule. The functionalizable nature of the presently disclosed materials is well suited for these purposes.

Some applications call for surfaces with low binding characteristics. Proteins and many other biomolecules (such as eukaryotic and microbial cells) can passively adsorb to polystyrene through hydrophobic or ionic interactions. Some surface-modified base materials have been developed to address this problem. Corning® Ultra Low Attachment (Corning Incorporated – Life Sciences, Acton, Massachusetts, United States of America) is a hydrogel-coated polystyrene. The hydrogel coating renders the surface neutral and hydrophilic, preventing the attachment of almost all cells. Vessels made from the surface have uses in preventing serum protein absorption, in preventing anchorage-dependent cells (MDCK, VERO, C6, and the like) from dividing, in selectively culturing tumor or virally transformed cells as

unattached colonies, in preventing stem cells from attachment-mediated differentiation, and in studying the activation and inactivation mechanisms of macrophages. NUNC MINISORP™ (Nalgene Nunc International, Naperville, Illinois, United States of America) is polyethylene-based product with low protein affinity and has uses for DNA probe and serum-based assays where non-specific binding is a problem.

For other applications base, materials have been modified to enhance their ability to adhere to cells and other biomolecules. NUNCLON Δ™ (Nalgene Nunc International) is a polystyrene surface treated by corona or plasma discharge to add surface carboxyl groups, rendering the material hydrophilic and negatively charged. The material has been used in the cell culture of a variety of cells. Polyolefin and polyester materials also have been treated to enhance their hydrophilicity and thereby become good surfaces for the adhesion and growth of cells (for example PERMANOX™ and THERMANOX™, also from Nalgene Nunc International). Base materials can be coated with poly-D-lysine, collagen or fibronectin to create a positively charged surface, which also can enhance cell attachment, growth and differentiation.

Further, other molecules can be absorbed to a microtiter-like plate. Nunc MAXISORP™ (Nalgene Nunc) is a modified polystyrene base that has a high affinity for polar molecules and is recommended for surfaces where antibodies need to be absorbed to the surface, as in the case of many ELISA assays. Surfaces also can be modified to interact with analytes in a more specific manner. Examples of such functional modifications include nickel-chelate modified surfaces for the quantification and detection of histidine-tagged fusion proteins and glutathione-modified surfaces for the capture of GST-tagged fusion proteins. Streptavidin-coated surfaces can be used when working with biotinylated proteins.

Some modified surfaces provide sites for the covalent attachment of various molecules or biomolecules. COVALINK™ NH Secondary Amine surface (Nalgene Nunc International) is a polystyrene surface covered with secondary amines which can bind proteins and peptides through their carboxyl groups via carbodimide chemistry or bind DNA through the formation

of a 5' phosporamidiate bond (again using carbodimide chemistry). Other molecules, carbohydrates, hormones, small molecules and the like, containing or modified to contain carboxylate groups also can be bound to the surface. Epoxide is another useful moiety for covalently linking groups to surfaces. Epoxide modified surfaces have been used to create DNA chips *via* the reaction of amino-modified oligonucleotides with surfaces. Surfaces with immobilized oligonucleotides can be of use in high throughput DNA and RNA detection systems and in automated DNA amplification applications.

Other uses for microtiter plates are directed toward modifying the surface to make it more hydrophobic, rendering it more compatible with organic solvents or to reduce the absorption of drugs, usually small organic molecules. For example, Total Drug Analysis assays generally rely on using acetonitrile to precipitate proteins and salts from a plasma or serum sample. The drug being assayed must remain in solution for subsequent quantification. Organic solvent-compatible microtiter plates also have uses as high performance liquid chromatography (HPLC) or liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) prep devices and as combinatorial chemistry or parallel synthesis reaction vessels (either for solution-based or solid phase chemistries). Examples of surfaces for these types of uses include MULTICHEM™ microplates (Whatman, Inc., Florham Park, New Jersey, United States of America) and MULTISCREEN® Solvinert (Millipore, Billerica, Massachusetts, United States of America).

XIII. Method for Using a Functionalized Perfluoropolyether Network as a Gas Separation Membrane

The presently disclosed subject matter provides for the use of a functionalized perfluoropolyether (PFPE) network as a gas separation membrane. In some embodiments, the functionalized PFPE network is used as a gas separation membrane to separate gases selected from the group consisting of CO_2, methane, hydrogen, CO, CFCs, CFC alternatives, organics, nitrogen, methane, H_2S, amines, fluorocarbons, fluoroolefins, and O_2. In some embodiments, the functionalized PFPE network is used to separate gases in a water purification process. In some embodiments, the

gas separation membrane comprises a stand-alone film. In some embodiments, the gas separation membrane comprises a composite film.

In some embodiments, the gas separation membrane comprises a co-monomer. In some embodiments, the co-monomer regulates the permeability properties of the gas separation membrane. Further, the mechanical strength and durability of such membranes can be finely tuned by adding composite fillers, such as silica particles and others, to the membrane. Accordingly, in some embodiments, the membrane further comprises a composite filler. In some embodiments, the composite filler comprises silica particles.

EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

General Considerations

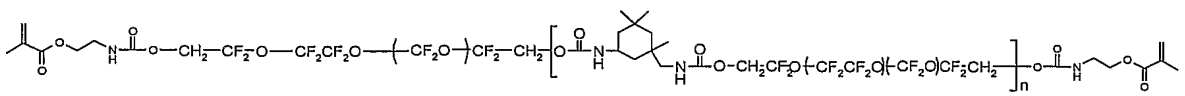
A PFPE microfluidic device has been previously reported by Rolland, J. et al. *JACS* **2004**, *126*, 2322-2323, which is incorporated herein by reference in its entirety. The specific PFPE material disclosed in Rolland, J., et al., was not chain extended and therefore did not possess the multiple hydrogen bonds that are present when PFPEs are chain extended with a diisocyanate linker. Nor did the material posses the higher molecular weights between crosslinks that are needed to improve mechanical properties such as modulus and tear strength which are critical to a variety of microfluidics applications. Furthermore, this material was not functionalized to incorporate various moieties, such as a charged species, a biopolymer, or a catalyst.

Herein is described a variety of methods to address these issues. Included in these improvements are methods which describe chain extension,

improved adhesion to multiple PFPE layers and to other substrates such as glass, silicon, quartz, and other polymers as well as the ability to incorporate functional monomers capable of changing wetting properties or of attaching catalysts, biomolecules or other species. Also described are improved methods of curing PFPE elastomers which involve thermal free radical cures, two-component curing chemistries, and photocuring using photoacid generators.

Example 1

A liquid PFPE precursor having the structure shown below (where n = 2) is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Thirdly, a smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE precursor across a glass slide. The Slide is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 120 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the fully cured PFPE smooth layer on the glass slide and allowed to heat at 120 °C for 15 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science.* **2000,** *288*, 113-6.



Example 2

Thermal Free Radical

Glass

A liquid PFPE precursor encapped with methacrylate groups is blended with 1 wt% of 2,2-Azobisisobutyronitrile and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in an oven at 65 °C for 20 hours under nitrogen purge. The cured layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of a clean glass slide and fluids are introduced through the inlet holes.

Example 3

Thermal Free Radical – Partial Cure

Layer to Layer Adhesion

A liquid PFPE precursor encapped with methacrylate groups is blended with 1 wt% of 2,2-Azobisisobutyronitrile and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. Thirdly, a smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE precursor across a glass slide. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 10 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes

and outlet holes are punched using a luer stub. The bonded layers are then placed on the partially cured PFPE smooth layer on the glass slide and allowed to heat at 65 °C for 10 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

Example 4

Thermal Free Radical – Partial Cure

Adhesion to Polyurethane

A photocurable liquid polyurethane precursor containing methacrylate groups is blended with 1 wt% of 2,2-Azobisisobutyronitrile and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of approximately 3 mm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of approximately 20 μm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. Thirdly, a smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE precursor across a glass slide. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 10 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the partially cured PFPE smooth layer on the glass slide and allowed to heat at 65 °C for 10 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

Example 5

Thermal Free Radical –Partial Cure

Adhesion to Silicone-containing Polyurethane

A photocurable liquid polyurethane precursor containing PDMS blocks and methacrylate groups is blended with 1 wt% of 2,2-Azobisisobutyronitrile and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of approximately 3 mm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of approximately 20 μm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. Thirdly, a smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE precursor across a glass slide. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 10 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the partially cured PFPE smooth layer on the glass slide and allowed to heat at 65 °C for 10 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

Example 6

Thermal Free Radical – Partial Cure

Adhesion to PFPE-PDMS block copolymer

A liquid precursor containing both PFPE and PDMS blocks encapped with methacrylate groups is blended with 1 wt% of 2,2-Azobisisobutyronitrile and poured over a microfluidics master containing 100-μm features in the

shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of approximately 3 mm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of approximately 20 μm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. Thirdly, a smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE precursor across a glass slide. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 10 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the partially cured PFPE smooth layer on the glass slide and allowed to heat at 65 °C for 10 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

Example 7

Thermal Free Radical – Partial Cure

Glass Adhesion

A liquid PFPE precursor encapped with methacrylate groups is blended with 1 wt% of 2,2-Azobisisobutyronitrile and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. The partially cured layer is removed from the wafer and inlet holes are punched using a luer stub. The layer is then placed on top of a glass slide treated with a silane coupling agent, trimethoxysilyl propyl methacrylate. The layer is then placed in an oven and allowed to heat at 65 °C for 20 hours,

permanently bonding the PFPE layer to the glass slide. Small needles can then be placed in the inlets to introduce fluids.

Example 8

Thermal Free Radical – Partial Cure

PDMS Adhesion

A liquid poly(dimethylsiloxane) precursor poured over a microfluidics master containing 100-μm features in the shape of channels. The wafer is then placed in an oven at 80 °C for 3 hours. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of liquid PFPE precursor encapped with methacrylate units at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. The PDMS layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then treated with an oxygen plasma for 20 minutes followed by treatment with a silane coupling agent, trimethoxysilyl propyl methacrylate. The treated PDMS layer is then placed on top of the partially cured PFPE thin layer and heated at 65 °C for 10 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the partially cured PFPE smooth layer on the glass slide and allowed to heat at 65 °C for 10 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

Example 9

Thermal Free Radical

PDMS Adhesion using SYLGARD 184® and functional PDMS.

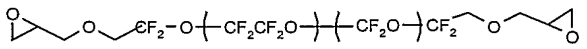
A liquid poly(dimethylsiloxane) precursor is designed such that it can be part of the base or curing component of SYLGARD 184®. The precursor contains latent functionalities such as epoxy, methacrylate, or amines and is mixed with the standard curing agents and poured over a microfluidics master containing 100-μm features in the shape of channels. The wafer is then

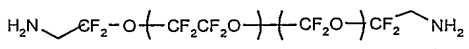
placed in an oven at 80 °C for 3 hours. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of liquid PFPE precursor encapped with methacrylate units at 3700 rpm for 1 minute to a thickness of approximately 20 μm. The wafer is then placed in an oven at 65 °C for 2-3 hours under nitrogen purge. The PDMS layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The PDMS layer is then placed on top of the partially cured PFPE thin layer and heated at 65 °C for 10 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the partially cured PFPE smooth layer on the glass slide and allowed to heat at 65 °C for 10 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

Example 10

Epoxy/Amine

A two-component liquid PFPE precursor system such as shown below containing a PFPE diepoxy and a PFPE diamine are blended together in a stochiometric ratio and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in an oven at 65 °C for 5 hours. The cured layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of a clean glass slide and fluids are introduced through the inlet holes.



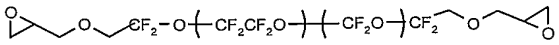


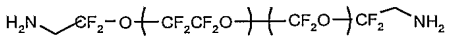
Example 11

Epoxy/Amine – Excess

Adhesion to Glass

A two-component liquid PFPE precursor system such as shown below containing a PFPE diepoxy and a PFPE diamine are blended together in a 4:1 epoxy:amine ratio such that there is an excess of epoxy and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in an oven at 65 °C for 5 hours. The cured layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of a clean glass slide that has been treated with a silane coupling agent, aminopropyltriethoxy silane. The slide is then heated at 65 °C for 5 hours to permanently bond the device to the glass slide. Fluids are then introduced through the inlet holes.





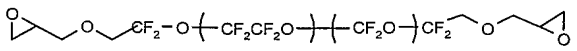
Example 12

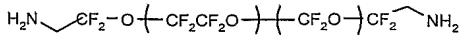
Epoxy/Amine – Excess

Adhesion to PFPE layers

A two-component liquid PFPE precursor system such as shown below containing a PFPE diepoxy and a PFPE diamine are blended together in a 1:4 epoxy:amine ratio such that there is an excess of amine and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. Separately, a second master containing 100-μm features in the shape of channels is coated with a small drop of liquid PFPE precursors blended in a 4:1 epoxy:amine ratio such that there is an excess of epoxy units and spin coated at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in an oven at 65 °C for 5 hours. The thick layer is then removed, trimmed, and inlet holes are punched through it using a luer stub.

The thick layer is then placed on top of the cured PFPE thin layer and heated at 65 °C for 5 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane and heated in an oven at 65 °C for 5 hours to adhere the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.





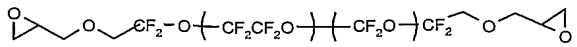
Example 13

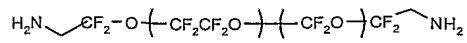
Epoxy/Amine – Excess

Adhesion to PDMS layers

A liquid poly(dimethylsiloxane) precursor is poured over a microfluidics master containing 100-μm features in the shape of channels. The wafer is then placed in an oven at 80 °C for 3 hours. Separately, a second master containing 100-μm features in the shape of channels is coated with a small drop of liquid PFPE precursors blended in a 4:1 epoxy:amine ratio such that there is an excess of epoxy units and spin coated at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in an oven at 65 °C for 5 hours. The PDMS layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then treated with an oxygen plasma for 20 minutes followed by treatment with a silane coupling agent, aminopropyltriethoxy silane. The treated PDMS layer is then placed on top of the PFPE thin layer and heated at 65 °C for 10 hours to adhere the two layers. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with aminopropyltriethoxy silane and allowed to heat at 65 °C for 10 hours. Small

needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.



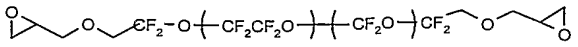


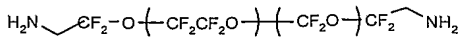
Example 14

Epoxy/Amine – Excess

Adhesion to PFPE layers, Attachment of a Biomolecule

A two-component liquid PFPE precursor system such as shown below containing a PFPE diepoxy and a PFPE diamine are blended together in a 1:4 epoxy:amine ratio such that there is an excess of amine and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. Separately, a second master containing 100-μm features in the shape of channels is coated with a small drop of liquid PFPE precursors blended in a 4:1 epoxy:amine ratio such that there is an excess of epoxy units and spin coated at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in an oven at 65 °C for 5 hours. The thick layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The thick layer is then placed on top of the cured PFPE thin layer and heated at 65 °C for 5 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane and heated in an oven at 65 °C for 5 hours to adhere the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6. An aqueous solution containing a protein functionalized with a free amine is then flowed through the channel which is lined with unreacted epoxy moieties, in such a way that the channel is then functionalized with the protein.



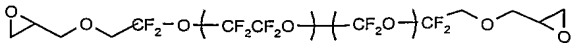


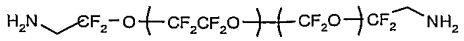
Example 15

Epoxy/Amine – Excess

Adhesion to PFPE layers, Attachment of a Charged Species

A two-component liquid PFPE precursor system such as shown below containing a PFPE diepoxy and a PFPE diamine are blended together in a 1:4 epoxy:amine ratio such that there is an excess of amine and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. Separately, a second master containing 100-μm features in the shape of channels is coated with a small drop of liquid PFPE precursors blended in a 4:1 epoxy:amine ratio such that there is an excess of epoxy units and spin coated at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in an oven at 65 °C for 5 hours. The thick layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The thick layer is then placed on top of the cured PFPE thin layer and heated at 65 °C for 5 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane and heated in an oven at 65 °C for 5 hours to adhere the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6. An aqueous solution containing a charged molecule functionalized with a free amine is then flowed through the channel which is lined with unreacted epoxy moieties, in such a way that the channel is then functionalized with the charged molecule.



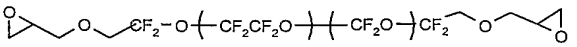


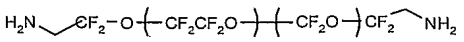
Example 16

Epoxy/Amine – Partial Cure

Adhesion to glass

A two-component liquid PFPE precursor system such as shown below containing a PFPE diepoxy and a PFPE diamine are blended together in a stochiometric ratio and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in an oven at 65 °C for 0.5 hours such that it is partially cured. The partially cured layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 5 hours such that it is adhered to the glass slide. Small needles can then be placed in the inlets to introduce fluids.





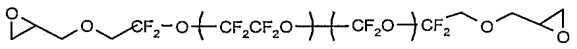
Example 17

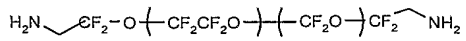
Epoxy/Amine – Partial Cure

Layer to Layer Adhesion

A two-component liquid PFPE precursor system such as shown below containing a PFPE diepoxy and a PFPE diamine are blended together in a stochiometric ratio and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in an oven at 65 °C for 0.5 hours such that it is partially cured. The partially

cured layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursors over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in an oven at 65 °C for 0.5 hours such that it is partially cured. The thick layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 1 hour to adhere the two layers. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 10 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.





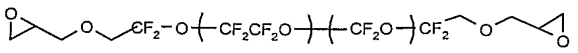
Example 18

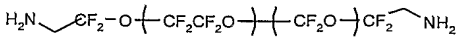
Epoxy/Amine – Partial Cure

PDMS Adhesion

A liquid poly(dimethylsiloxane) precursor is poured over a microfluidics master containing 100-μm features in the shape of channels. The wafer is then placed in an oven at 80 °C for 3 hours. The cured PDMS layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then treated with an oxygen plasma for 20 minutes followed by treatment with a silane coupling agent, aminopropyltriethoxy silane. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of liquid PFPE precursors mixed in a stochiometric ratio at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in an oven at 65 °C for 0.5 hours. The treated

PDMS layer is then placed on top of the partially cured PFPE thin layer and heated at 65 °C for 1 hour. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with aminopropyltriethoxy silane and allowed to heat at 65 °C for 10 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

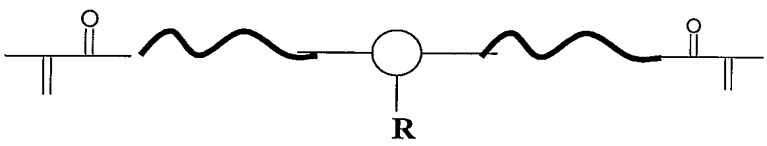




Example 19

Photocuring with Latent Functional Groups Available Post Cure Adhesion To Glass

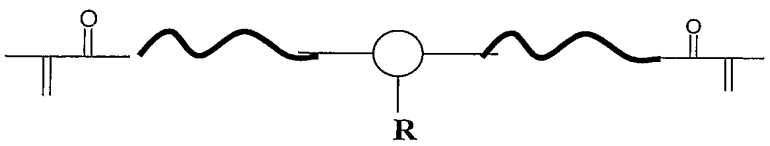
A liquid PFPE precursor having the structure shown below (where R is an epoxy group, the curvy lines are PFPE chains, and the circle is a linking molecule) is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The fully cured layer is then removed from the master and inlet holes are punched using a luer stub. The device is placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids.



Example 20

Photocuring with Latent Functional Groups Available Post Cure Adhesion to PFPE

A liquid PFPE precursor having the structure shown below (where R is an epoxy group), the curvy lines are PFPE chains, and the circle is a linking molecule) is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The fully cured layer is then removed from the master and inlet holes are punched using a luer stub. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor (where R is an amine group) over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

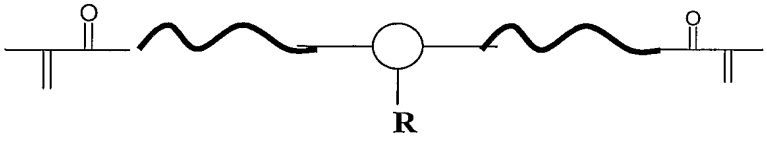


Example 21

Photocuring w/ latent functional groups available post cure

Adhesion to PDMS

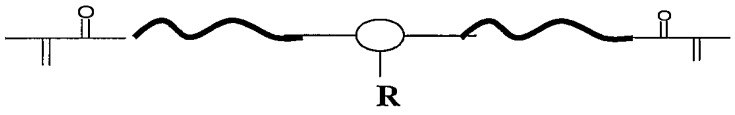
A liquid poly(dimethylsiloxane) precursor is poured over a microfluidics master containing 100-μm features in the shape of channels. The wafer is then placed in an oven at 80 °C for 3 hours. The cured PDMS layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then treated with an oxygen plasma for 20 minutes followed by treatment with a silane coupling agent, aminopropyltriethoxy silane. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor (where R is an epoxy) over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker PDMS layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.



Example 22

Photocuring with Latent Functional Groups Available Post Cure Attachment of Biomolecule

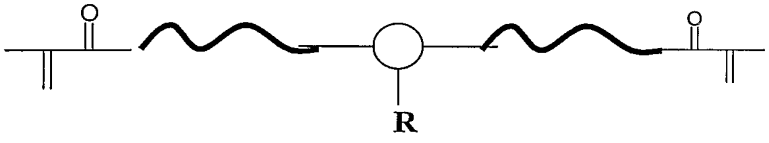
A liquid PFPE precursor having the structure shown below (where R is an amine group), the curvy lines are PFPE chains, and the circle is a linking molecule) is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The fully cured layer is then removed from the master and inlet holes are punched using a luer stub. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor (where R is an epoxy group) over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6. An aqueous solution containing a protein functionalized with a free amine is then flowed through the channel which is lined with unreacted epoxy moieties, in such a way that the channel is then functionalized with the protein.



Example 23

Photocuring with Latent Functional Groups Available Post Cure Attachment of Charged Species

A liquid PFPE precursor having the structure shown below (where R is an amine group), the curvy lines are PFPE chains, and the circle is a linking molecule) is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The fully cured layer is then removed from the master and inlet holes are punched using a luer stub. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor (where R is an epoxy group) over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000**, *288*, 113-6. An aqueous solution containing a charged molecule functionalized with a free amine is then flowed through the channel which is lined with unreacted epoxy moieties, in such a way that the channel is then functionalized with the charged molecule.

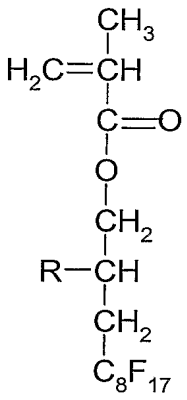


Example 24

Photocuring with Functional Monomers Available Post Cure

Adhesion to Glass

A liquid PFPE dimethacrylate precursor or a monomethacrylate PFPE macromonomer is blended with a monomer having the structure shown below (where R is an epoxy group) and blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The fully cured layer is then removed from the master and inlet holes are punched using a luer stub. The device is placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids.



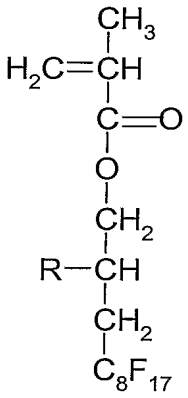
Example 25

Photocuring with Functional Monomers Available Post Cure

Adhesion to PFPE

A liquid PFPE dimethacrylate precursor is blended with a monomer having the structure shown below (where R is an epoxy group) and blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365)

for 10 minutes under a nitrogen purge. The fully cured layer is then removed from the master and inlet holes are punched using a luer stub. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor plus functional (where R is an amine group) over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

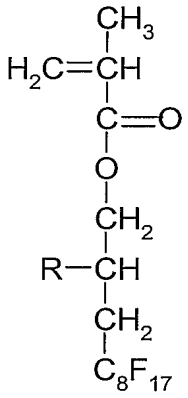


Example 26

Photocuring with Functional Monomers Available Post Cure Adhesion to PDMS

A liquid poly(dimethylsiloxane) precursor is poured over a microfluidics master containing 100-μm features in the shape of channels. The wafer is then placed in an oven at 80 °C for 3 hours. The cured PDMS layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then treated with an oxygen plasma for 20 minutes followed by

treatment with a silane coupling agent, aminopropyltriethoxy silane. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of a liquid PFPE dimethacrylate precursor plus functional monomer (where R is an epoxy) plus a photoinitiator over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker PDMS layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science.* **2000,** *288*, 113-6.

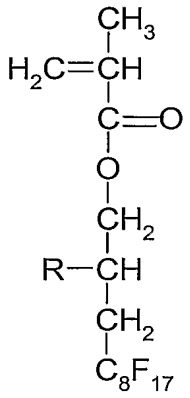


Example 27

Photocuring with Functional Monomers Available Post Cure Attachment of a Biomolecule

A liquid PFPE dimethacrylate precursor is blended with a monomer having the structure shown below (where R is an amine group) and blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm.

The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The fully cured layer is then removed from the master and inlet holes are punched using a luer stub. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor plus functional (where R is an epoxy group) over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6. An aqueous solution containing a protein functionalized with a free amine is then flowed through the channel which is lined with unreacted epoxy moieties, in such a way that the channel is then functionalized with the protein.

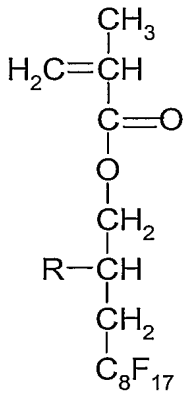


Example 28

Photocuring with Latent Functional Groups Available Post Cure Attachment of Charged Species

A liquid PFPE dimethacrylate precursor is blended with a monomer

having the structure shown below (where R is an amine group) and blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The fully cured layer is then removed from the master and inlet holes are punched using a luer stub. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor plus functional (where R is an epoxy group) over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 65 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a glass slide treated with a silane coupling agent, aminopropyltriethoxy silane, and allowed to heat at 65 °C for 15 hours permanently bonding the device to the glass slide. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6. An aqueous solution containing a charged molecule functionalized with a free amine is then flowed through the channel which is lined with unreacted epoxy moieties, in such a way that the channel is then functionalized with the charged molecule.



Example 29

Fabrication of a PFPE Microfluidic Device using Sacrificial Channels

A smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE dimethacrylate precursor across a glass slide. The Slide is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. A scaffold composed of poly(lactic acid) in the shape of channels is laid on top of the flat, smooth layer of PFPE. A liquid PFPE dimethacrylate precursor is with 1 wt% of a free radical photoinitiator and poured over the scaffold. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The apparatus is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The device is then heated at 150 °C for 24 hours to degrade the poly(lactic acid) thus revealing voids left in the shape of channels.

Example 30

Adhesion of a PFPE Device to Glass using 185-nm Light

A liquid PFPE dimethacrylate precursor is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 120 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes

are punched using a luer stub. The bonded layers are then placed on a clean, glass slide in such a way that it forms as seal. The apparatus is exposed to 185 nm UV light for 20 minutes, forming a permanent bond between the device and the glass. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.

Example 31

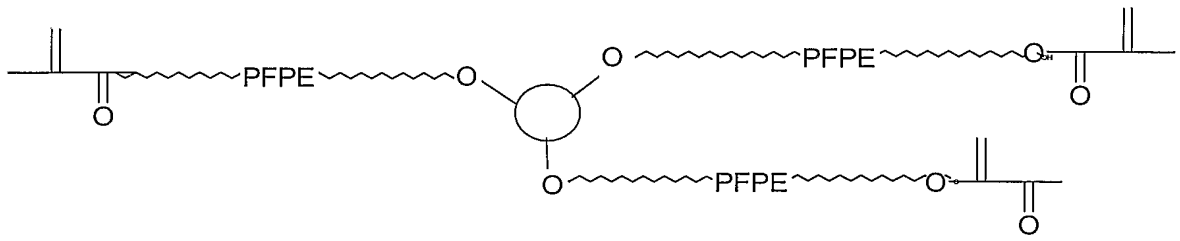
"Epoxy Casing" Method to Encapsulate Devices

A liquid PFPE dimethacrylate precursor is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 120 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a clean, glass slide in such a way that it forms as seal. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6. The entire apparatus is then encased in a liquid epoxy precursor which is poured over the device allowed to cure. The casing serves to mechanically bind the device the substrate.

Example 32

Fabrication of a PFPE Device from a Three-Armed PFPE Precursor

A liquid PFPE precursor having the structure shown below (where the circle represents a linking molecule) is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Thirdly a smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE precursor across a glass slide. The Slide is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 120 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the fully cured PFPE smooth layer on the glass slide and allowed to heat at 120 °C for 15 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.



Example 33

Photocured PFPE/PDMS Hybrid

A master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE dimethacrylate precursor containing photoinitiator over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. A PDMS dimethacrylate containing photoinitiator is then poured over top of the thin PFPE layer to a thickness of 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The hybrid device is then placed on a glass slide and a seal is formed. Small needles can then be placed in the inlets to introduce fluids.

It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.